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Early maturity in Canadian spring wheat as influenced by genes on group 5
chromosomes.

By

Shaheen Rahman 

A thesis submitted to the Faculty of Graduate Studies and

Research in partial fulfillment of the requirements

For the degree of Master of Science

In

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, of a thesis entitled : **Early maturity in Canadian spring wheat as influenced by genes on group 5 chromosomes**, Submitted by **Shaheen Rahman** in partial fulfillment of the requirements for the degree of Master of Science in Plant Science.

Abstract

The genetic effect of group 5 chromosomes on heading and maturity in spring wheat (*Triticum aestivum* L.) in days and heat units was investigated. A series of chromosome 5A, 5B and 5D substitution lines known to be polymorphic for vernalization (*Vrn*) genes in Cadet and Rescue backgrounds was field evaluated at Edmonton over a wide range of seeding dates for three years.

The full set of substitution lines exhibited a wide range of maturity requirements typical of that found in Canadian spring wheat cultivars. Chromosome 5B substitution lines showed a greater genetic effect by reducing the time necessary for heading and maturity compared to lines 5A and 5D. A putative winter type carrying *vrn1*, 3 and 4 in a Cadet background headed in two of three trials despite absence of cool spring conditions, presumably through vernalization at higher temperatures. Substitution of 5D chromosome generally did not significantly affect days to heading or maturity in either Cadet or Rescue backgrounds. Similar trends with respect to chromosome substitution effects were observed when lines were evaluated on the basis of heat unit accumulation and photon flux accumulated in the field.

The heading and maturity requirements of the check cultivars and the substitution lines under controlled environment conditions were similar to that observed in the field, and the relative rankings remained the same. The search for RAPD markers for earliness genes on chromosome 5D was unsuccessful. The earliness segregation pattern of F₂ populations polymorphic for days to maturity suggested complex inheritance in one of the two crosses.

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Abbreviations

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of variance
bp	base pair
BCA	Bicinchoninic acid
BSA	Bulk segregant analysis
cm	centimeter
CWRS	Canada Western Red Spring
DH	Days to heading
DHM	Days from heading to maturity
DM	Days to maturity
DNA	deoxy ribonucleic acid
dNTP	deoxy ribonucleotide triphosphates
EDTA	ethylenediaminetetraacetic acid
F ₁	First filial generation
F ₂	Second filial generation
FLC	Flowering Locus C
FRI	FRIGIDA
GA	Gibberellic acid
GDD	Growing degree-days
GLM	general linear model
hr	hour
KAO	ent-Kaurenoic acid oxidase
kda	kilodalton
KO	ent-Kaurene oxidase
mA	milliampere
min	minute
ml	milliliter
mM	millimolar

MOP	Maximum optimum photoperiod
ng	nanogram
PCR	Polymerase Chain Reaction
PF	photon flux
PTU	photo-thermal units
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SAS	Statistical Analysis Software
SD	Standard deviation
SDS	Sodium Dodecyl Sulfate (Sodium lauryl sulfate)
SE	Standard Error
sec	second
UV	Ultra-violet light
°C	Degree Celsius
μg	microgram
μl	microlitre
μM	micromolar

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Chapter 1. Introduction

1.1. Introduction

The short growing season in the northern prairies is the primary limiting factor for the consistent production of high quality spring wheat. A breeding effort for early maturing wheat (*Triticum aestivum* L.) is necessary to produce wheat for the Parkland regions of Western Canada. Time to maturity consists of the sum of several developmental periods. Wong and Baker (1986) suggested that through phenological and yield component studies, it might be possible to balance the duration of developmental periods. In doing so, a more desirable combination of earliness and high yield might be achieved.

A better understanding of the genetic control of the maturity in spring wheat as influenced by environmental factors (vernalization requirement, photoperiod and temperature response, growing degree-days and photon flux requirements) or simply “days” and its genetic constituents will guide breeders in targeting crosses to enhance early maturity. It will also improve our understanding of regional adaptation more precisely and accurately. Early maturing cultivars with differing maturities are essential to exploit the considerable environmental variability throughout the prairies. Early maturing wheat crops are also essential for the reliability and ease of crop management under a diversified production system, the need for wheat as a break in crop rotation from barley and canola, and the need for early maturing, higher yielding wheat in areas with a short growing season.

Agronomically, wheat cultivars are divided into two distinct groups, winter and spring types. This growth habit of wheat is governed by vernalization (*Vrn*) genes (Pugsley 1971, 1972). The spring growth habit is dominant over winter growth habit, and the presence of a dominant allele of any of the *Vrn* genes effectively inhibits the expression of the winter growth habit (Brulé-Babel and Fowler 1988). In reality, most spring wheats respond to a low temperature treatment by decreasing the period necessary to flowering. Also, different combinations of vernalization genes affect the time to flowering. In spring wheat, the number of *Vrn* genes present affects duration of time to seed maturation. *Vrn* genes 1, 3 and 4 are more effective than *Vrn*1 and 5 in fulfilling the

vernalization requirement in spring wheat. Five major genes governing vernalization requirement: *Vrn1*, *Vrn2*, *Vrn3* (Pugsley 1971, 1972), *Vrn4* (Pugsley 1973), and *Vrn5* (Law 1966) have been identified. The effects of these major genes in controlling variation for ear emergence time in hexaploid wheat are well characterized (Crumpacker and Allard 1960, Islam-Faridi et al. 1996; Stelmakh 1993). Homeoallelic genes for vernalization requirement *Vrn1*, *Vrn4* and *Vrn3* are located on the long arms of chromosomes 5A, 5B, and 5D respectively (Stelmakh 1998). Another gene affecting the progression to flowering has been reported (*Vrn5*) which is located on the short arm of chromosome 7B of the substitution line of Chinese Spring (Law 1966; Islam-Faridi et al. 1996).

Vernalization genes and their interaction with the other photoperiodic responsive genes (Jedel et al. 1986), as well as “earliness *per se*” genes (perhaps controlling the length of the basic vegetative stage) and putative temperature mediated “developmental rate” genes, all complicate our understanding of the precise genetic control of early maturity. Wheat is normally classified as a quantitative long day plant, (Bernier 1988) but some cultivars also flower under short day and long night conditions while other types develop only slowly under short days (Law et al. 1976). Various estimates of the number of genes controlling photoperiod responses (Pugsley 1965; Klaimi and Qualset 1973) indicated the involvement of two genes. Similarly, the day length insensitiveness of the Mexican semi-dwarf Sonora 64 was shown to be determined by at least two major genes, *Ppd1* and *Ppd2*, located on chromosomes 2B and 2D, both of which promote early maturity (Keim et al. 1973).

The chromosomal location of the *Vrn* genes has been determined (Pugsley 1971, 1972, 1973; Law 1966), and this information can be exploited in determining the effect of these genes on growth and development of wheat cultivars. The aneuploid series developed in common wheat, along with secondary aberrations such as telocentric and isochromosomes (Sears 1953) provides a means by which each of the chromosomes may be replaced by the homeologous chromosomes of other varieties or species. These substitution lines carrying different chromosomes can be compared in replicated field trials. Since each substituted chromosome is present in a more or less uniform genetic

background, it may be possible to determine the effect of the genes present on these chromosomes on quantitative and qualitative characters (Kuspira and Unrau 1957).

The molecular identification of markers associated with *Vrn* or earliness genes could help breeders in characterization and screening of cultivars and breeding lines. The Random Amplified Polymorphic DNA (RAPD) technique has been used to obtain markers associated with agronomic characters (Laroche et al. 2000). The identification of segregating markers in F₂ can be exploited to obtain markers associated with either *Vrn* gene(s) or 'earliness per se' genes. The use of Bulk Segregant Analysis (BSA, Michelmore et al. 1991) could possibly increase the selection efficiency.

The present study was undertaken to determine the range of maturity in current Canadian spring wheat cultivars under different sowing dates. Similarly, the effect of substituting chromosomes 5A, 5B or 5D in either Rescue or Cadet background was also determined. A number of criteria (days, growing degree-days, photo-thermal units and photon flux accumulation) were used to determine the range of heading and maturity of the check cultivars (Prelude, Park, Katepwa and Columbus), and the chromosome substituted lines, and the effect of chromosome (gene) introduction on these criteria. Another part of the study was aimed at finding molecular marker(s) associated with earliness through the use of RAPD molecular markers. The identification of such markers could facilitate the characterization of Canadian germplasm, and screening early breeding lines.

Genetic analysis of naturally occurring early- and late- flowering ecotypes of *Arabidopsis* have been used to identify several genes influencing flowering time (Aukerman and Amasino 1996). Their gene product has recently been identified and was reported to be involved in the vernalization response (Sheldon et al. 2000). The identification of these genes and their characterization can provide some insight into the molecular basis of vernalization response.

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Chapter 2. Literature review

2.1. Introduction

Spring wheat (*Triticum aestivum* L.) is the major crop species grown on the Canadian prairies. Common wheat is an allo-hexaploid ($2n=6x=42$) with a chromosome number of 42, and three genomes, designated A, B and D. Hexaploid wheat is believed to have evolved from a prototype diploid species that underwent a divergent evolution to give three progenitor diploid species, which later intercrossed to give rise to the hexaploids. Through cytogenetic studies, it has been established that common wheat contains the full set of chromosomes from *Triticum turgidum* (genome AABB). This has led to the conclusion that common wheat evolved from the hybridization of *T. turgidum* with *T. tauschii*, which contributed the D genome (McFadden and Sears 1946).

More than 90% of Canadian wheat is produced in the Prairie provinces of Manitoba, Saskatchewan and Alberta. The number of hectares utilized for wheat production in Alberta in 1999-2000 was 2.5 million (Statistics Canada, 2000). Wheat production continues to dominate the majority of the seeded area followed by canola, barley, corn and field pea in the prairie provinces. The production of total spring wheat was 20.9 million tons in 1999 and 19.4 million tons in 2000 (Statistics Canada, 2001). Over half of the wheat grown in the Canadian prairies is of the Canada Western Red Spring wheat (CWRS) type, while approximately 25% is Amber Durum (Statistics Canada, 2000).

The prairie region has long, cold winters, and short hot summers. On the average about 105 frost-free days are available in the Parkland area for growing crops. There is usually limited precipitation range, and droughts are common. A short growing season from early April to September limits the production to early maturing cultivars. The development of early maturing, high quality spring wheat thus continues to challenge Canadian plant breeders (DePauw et al. 1995). One of the most difficult problems in wheat breeding is the need to combine early maturity with high productivity. There are many advantages of growing wheat in the prairie agro-ecosystem: (1) its reliability, (2) ease of management under a diversity of production systems, (3) potential for high yields

under cooler and wetter conditions, and (4) tolerance to a wide range of biological and environmental stresses (Briggs 1987).

Most wheat cultivars are divided into two distinct groups: winter and spring types and both groups may express sensitivity or insensitivity to photoperiod. Martinic (1973) described three classifications of wheat based upon vernalization requirements: spring types that are non-responsive to cold treatment (no cold requirement); winter types with a vernalization requirement; and intermediate types (semi-winter, alternative, facultative etc.) that have a range of intermediate responses. Vernalization responsive (*Vrn*) genes control qualitative differences in the growth habit through the modulation of vernalization requirement, although other genetic control systems may also be involved, such as day-length sensitivity genes (Stelmakh 1998).

2.2. Effect of environmental factors on phenological development

2.2.1. Response to vernalization

Wheat genotypes vary widely in vernalization response (Halloran 1975; Jedel et al. 1986) and the genetic control of the vernalization mechanism is complex (Flood and Halloran 1984; Major and Whelan 1985). Chouard (1960) defined vernalization as ‘the acquisition, or acceleration, or the ability to flower by a chilling treatment’ i.e., the promotion of flowering by naturally or artificially applied periods of extended low temperatures, while according to Purvis (1961) this is a physiological process that enables plants to respond to the appropriate temperatures that induce flowering. The presence or absence of the vernalization requirement is an important factor in determining the effective length and temperature required for plant development. The optimum temperature for vernalization is around 7°C but vernalization can occur at any temperature ranging from 0° to 15°C (Terzioglu 1988).

Perception of the cold temperature occurs in the shoot tip, which includes the apex and immature leaves. When the vernalization requirement has been partially or completely fulfilled, the vegetative apex becomes physiologically responsive to photoperiodic stimuli necessary for flower initiation (Cooper 1956; Marcellos and Single 1971). Vernalization affects the time to floral initiation, leaf number, the timing of other growth stages up to the emergence of the flag leaf, and the number of tillers produced

(Levy and Peterson 1972; Syme 1973; Jedel et al. 1986). It is well documented in cereals that vernalization hastens the termination of vegetative growth, and initiates the reproductive stage as the morphology of the apex changes from single ridge to double ridge thereby initiating the reproductive structures (Purvis 1934; Fisher 1973; Wiegand et al. 1981). It is assumed that there is a strong relationship between the time from sowing to floral initiation and from sowing to spike emergence (Flood and Halloran 1986).

Genes for vernalization response have played an important role in the adaptation of common wheat through their influence on heading time. Five major genes governing vernalization requirement *Vrn1*, *Vrn2*, *Vrn3* (Pugsley 1971; 1972), *Vrn4* (Pugsley 1973), and *Vrn5* (Law 1966) have been identified. The major genes controlling variation in the ear emergence time in hexaploid wheat (*Vrn1*, *Vrn3*, *Vrn4*) are now well characterized. These homoeoallelic genes for vernalization requirement are located on the long arms of chromosomes 5A, 5B, and 5D respectively, while *Vrn2* is located on chromosome 2B (Law et al. 1976; Stelmakh 1993; Stelmakh 1998). Another gene affecting the progression to flowering has been reported (*Vrn5*) which is located on the short arm of the chromosome 7B of the substitution line of Chinese Spring wheat (Law 1966; Islam-Faridi et al. 1996).

In wheat, the spring growth habit is dominant over the winter growth habit, and the presence of a dominant allele of any of the *Vrn* genes effectively inhibits the expression of the winter growth habit (Brulé-Babel and Fowler 1988). The geographic distribution of *Vrn* genes supports the idea that the *Vrn* loci have different breeding values (Stelmakh 1990). In theory, any spring wheat variety possess one or more dominant alleles for vernalization, and low temperature exposure is not required for flower induction or the production of fertile spikes. The presence of dominant alleles at one or more *Vrn* loci results in partial or complete inhibition of the vernalization requirement (Stelmakh, 1990). In contrast, winter wheat carries recessive alleles for all known vernalization genes and must be vernalized by exposure to low temperature before it can produce fertile spikes. In reality, most spring wheats will respond to a low temperature treatment by further decreasing the period necessary to form fertile spikes, but winter types have an obligate requirement of low temperature to produce fertile spikes. Although spring and winter cultivars differ by more than their vernalization

requirement, studies on the genetics of vernalization have often been labeled as growth habit studies (Klaimi and Qualset 1974). McKinney and Sando (1933) viewed the spring and winter habit as being differing degrees of earliness and lateness so that upon spring planting those plants that flowered within the season were termed spring and those that did not initiate flowering were termed winter. In studies of segregating progenies of crosses between spring and winter or spring and spring wheats, segregants have been classified into spring and winter types (Klaimi and Qualset 1974; Gotoh 1979). However, many researchers have noted continuous variation in the spring category and have split the spring segregants into classes based upon development (Gotoh 1979). Despite the limitation of phenological development being controlled by processes additional to vernalization, using earliness and lateness as quantifiers of vernalization response has allowed for the identification of the *Vrn* genes (Pugsley 1971; 1972; Gotoh 1979).

Aneuploid series studies have been used to locate the chromosomes on which the genes governing vernalization lie and to determine the multiple allelic and dosage dependent nature of the *Vrn* genes. It have been documented that different combinations of vernalization genes affect genotypes differently in time to flowering (Major and Whelan 1985; Roberts and Larson 1985) that enables breeding for favorable combinations resulting in suitable time to maturity to occur. The lines bearing the weakest (*Vrn2* and *Vrn5*) or recessive alleles respond more favorably to a low temperature treatment, but with concurrent decrease in yield potential (Aksel 1994). Vernalization genes 1, 3 and 4 have been shown to be more effective than *Vrn2* or 5 in fulfilling the vernalization requirement in spring wheat (Major and Whelan 1985; Roberts and Larson 1985). The strength of the vernalization response is measured by the time necessary to reach spike emergence or anthesis. According to Maystrenko (1980) the order of strength of the *Vrn* genes is *Vrn1*, *Vrn4*, *Vrn3*, *Vrn2* respectively. The most frequently involved chromosomes in the vernalization response are 5A and 5D, and less frequently 2B, 5B and 7B (Major and Whelan 1985). The relationship of vernalization effects suggest that the *Vrn* genes appear to act as non-complementary genes with classical (*Vrn1*) or incomplete (*Vrn3*) dominant epistasis (Stelmakh, 1993).

Wall and Cartwright (1974) concluded that vernalization and photoperiod response play an important role in the control of crop maturity and thereby are important factors governing yield. Increased dosage of the 5D chromosome (*Vrn3*) resulting in earlier flowering and decreased vernalization response has also been reported (Halloran 1967; Cahalan and Law 1979). The lines carrying *Vrn3* develop slowly in average environments (compared to lines containing *Vrn1*), resulting in a higher number of floral primordia, thus promoting potential yield increase. Thus, in moderate temperatures, they head later than the lines possessing *Vrn1*, but at increased temperature lines containing *Vrn3* head earlier, with the possibility of a higher yield potential (Stelmakh 1998).

According to Stelmakh (1987) *Vrn1* on chromosome 5A, *Vrn2* on 2B and *Vrn3* on 5D exists in *Triticum aestivum*, and *Vrn4* on 5B in cultivars like Gabo, Shortandinka, Milturum 553, Pirotrix 28, and *Vrn5* on 7B of Chinese spring substitution line of Hope. Spring cultivars possess one or more dominant genes in different combinations. The cultivars possessing *Vrn4* may also carry *Vrn1*, *Vrn2* and/or *Vrn3*. Stelmakh (1998) suggests about one-fourth of the spring cultivars contain the monogenic dominant *Vrn1* genotype, and up to one-half of them contain *Vrn1* and *Vrn2* in their genotype. The existence of *Vrn3* in combination with different alleles of the other loci was revealed less frequently, and the triple dominant genotype was not found in any of the commercial cultivars (Stelmakh 1998).

2.2.2. Response to temperature

Temperature is an environmental factor that influences the expression of phenological development. As a biological phenomenon, vernalization is unusual because while low temperatures slow down most biochemical processes, in the vernalization process, on the other hand, low temperature induces flowering (Purvis 1961). A close association between fulfilling vernalization requirement and the decline of a specific class of mRNA (from the *Wcs120* gene family) and protein accumulation was observed in wheat plants maintained at 4°C. Spring wheat cultivars not having a vernalization response were unable to maintain low temperature-induced genes in an up-regulated condition when exposed to 4°C. Molecular analysis showed that WCS120 proteins were expressed by spring- and winter-habit cultivars, but their level was very low in non-

vernalized plants, but increased rapidly due to vernalization. After a certain period, the level of the protein decreased, indicating the low-temperature response being a function of the degree and the duration of gene expression, and not being due to the activation of different sets of low-temperature genes. The *Wcs120* gene family mRNA level and the protein accumulation patterns indicate that low-temperature induced genes are down regulated once vernalization saturation is achieved in winter wheat. This was taken as an indication of the regulatory effect of vernalization genes over low-temperature gene expression in winter wheat (Fowler et al. 1996).

Genetic variation in wheat for responses to growth and temperature has recently been reported (Slafer and Rawson 1995). Temperature and photoperiod exhibit interaction in determining the rate of reproductive development in wheat. It is difficult in field studies to separate the influences of photoperiod and vernalization from temperature on growth rate and development because of the strong correlation between the length of the photoperiod and daily levels of solar radiation (Flood and Halloran 1983). For Chinese Spring wheat, the basic development rate was reported to be associated with chromosomes of homeologous group 2 of substituted Thatcher (Flood and Halloran 1983). These genes appear to confer sensitivity to growth temperature, but have different optima for maximal activity. It has been postulated that such genes could be either linked to vernalization and photoperiod responses, or can cause pleiotropic regulation of those responses (Flood and Halloran 1986). Whether the rates of all phases of development are changed in a genotype, or if a genotype can exhibit different rates in different phases of development, is unknown.

2.2.3. Response to photoperiod

Photoperiod is an important factor in determining time to heading and maturity (Davidson et al. 1985, Hoogendoorn 1985, Scarth et al. 1985), and increased or decreased photoperiod response depends upon the individual genotypes (Rahman and Wilson 1977). The leaves perceive the photoperiodic stimulus and a signal is transmitted to the apex (Gott et al. 1955; Evans 1987). Consequently, wheat plants cannot respond to photoperiod before the crops emerge (Hay and Kirby 1991). After emergence, plants can

perceive the photoperiod stimulus and can respond to it, and this perception can occur at any stage from seedling appearance to maturity.

Ear emergence is governed not only by *Vrn*, but also by photoperiod, and both can contribute equally to the variation in emergence time (Shindo and Sasakuma 1998). Wheat is a quantitative long-day plant and the development is faster in long days. Pinthus and Nerson (1984) examined the effect of short and long photoperiod on floral initiation and the initiation of the terminal spikelet, and apex development. They found that under a short photoperiod leaf primordia would continue to be formed until a genetically determined maximum number is reached. Once the genotypic critical leaf number is attained, the plants then switch to developing potential spikelet sites. As a result, the vegetative period ends and the spikelet production phase begins. A long photoperiod will restrict the number of spikelets formed in this period by promoting a terminal spikelet formation, whereas a short photoperiod will not initiate the development of the terminal spikelet for certain genotypes. Spike development is genotypically determined, but can also be affected by the length of the photoperiod (Scarth et al. 1985; Hoogendoorn 1985).

2.2.4. Photoperiod genes

Heading time of wheat is also under genetic control of photoperiod genes (*Ppd1*, *Ppd2* and *Ppd3*), located on group 2 homoeologous chromosomes 2D, 2B and 2A respectively (Welsh et al. 1973). Two loci, *Ppd1* and *Ppd2* have been identified which have major effects on photoperiod sensitivity (Scarth and Law 1984; Worland and Law 1986). These loci are located on the long arm of chromosome 2D and the short arm of 2B respectively, both of which promote earliness (Major and Whelan 1985); effect of *Ppd3* on day-length response has also been reported (Islam–Faridi 1988).

Pugsley (1966) found that the dominant alleles conferred insensitivity to photoperiod while the recessive alleles conferred sensitivity to photoperiod. Plants with insensitive alleles (*Ppd1* and *Ppd2*) have a faster growth rate of the floral apex than plants with sensitive alleles (*ppd1* and *ppd2*). The *Vrn* genes determine the division of wheat into spring and winter forms, while the *Ppd* genes govern the ability of wheat plants to grow and flower under short day conditions (Islam-Faridi et al. 1996). *Ppd1* is

an important dwarfing gene, which is insensitive to photoperiod but reduces plant height by shortening the life cycle (Worland and Law 1986). This *Ppd1* gene and its homoeologous *Ppd2* and *Ppd3* genes are very important in climatic adaptability of wheat (Worland 1996). *Ppd* genes that control the differences in photoperiodic response may be ascertained by comparing heading dates of vernalized genotypes under short- and long-day conditions. Photosensitive, fully recessive genotypes delay their heading when planted in short days, and the distinct differences are revealed under prolonged photoperiod. Photoinsensitive genotypes with dominant, photoperiod-sensitivity inhibitor alleles do not show such differences (Stelmakh 1998). The presence of dominant photoperiod alleles inhibits photosensitiveness to some extent, which leads to the absence of noticeable heading delay under short-day conditions. Similar to the vernalization gene system, photoperiod genes manifest incomplete dominance of insensitivity (or sensitivity inhibition), and the partial additivity of non-allelic genes is often conferred by dominant epistasis of the gene with more pronounced effect (Stelmakh 1998).

2.2.5. Early maturity

The main environmental factors affecting phasic development in wheat are photoperiod, vernalization and temperature (Slafer and Rawson 1994). Photoperiod and vernalization are usually considered to account for almost all of the differences between development rates of wheat cultivars. There might be another important factor in addition to photoperiod, vernalization and temperature, which could also affect phasic development. Hunt (1979) and Ford et al. (1981) described varietal differences in timing of ear emergence, which were independent of sensitivity to photoperiod and vernalization. These genetic differences are referred to as ‘earliness *per se*’, and could be used in breeding programs to shorten the life cycle of the plant irrespective of the prevailing day length and temperature conditions. Little is known about these ‘earliness *per se*’ genes or how they interact with the other environmental factors (Hoogendoorn 1985). Syme (1973) found that two cultivars, which had the same responses to photoperiod and vernalization but still had different ear emergence time, indicated genetic differences were responsible, even when the photoperiod and vernalization requirements were fully satisfied. Since it was presumed that these cultivars do not differ

in their response to temperature, these differences should therefore be ‘intrinsic’. Intrinsic factors affecting the length of the vegetative phase (the time to floral initiation) independent of any effects of photoperiod and vernalization have also been reported (Miura et al. 1994; Slafer 1996). This hypothetical factor has been given several names such as “intrinsic earliness” (Malse et al. 1989), “basic developmental rate” (Flood and Halloran 1984), and “narrow-sense earliness” (Kato et al. 1998). Miura et al. (1999) has shown that two genes on either arm of chromosome 3A determine “earliness *per se*” in wheat, while Sarma et al. (2000) reported the localization of a “earliness *per se*” along with *Vrn1* on chromosome 5A.

2.3. Heat Unit concept

2.3.1. Growing degree-days

Temperature is one of the primary factors influencing the developmental rate of wheat. Russelle et al. (1984) stated that plant growth and development show a temperature response, which results from the integrated effect of temperature on several physiological processes. Plants require energy in the form of heat to grow. The heat unit requirement is expressed as degrees of temperature. The theory behind this is that each plant has its own particular base temperature below which growth does not occur (Slafer and Rawson 1995). One way to study plant development in relation to the climate is the use of thermal time. Thermal time is defined as the time integral of daily temperature above the base temperature (Chalabi et al. 1988). This has led to the development of the growing degree-days (GDD) concept that is known today. The concept of growing degree-days assumes that the plant growth is related directly to the average daily temperature. The degree-days for each day are added together, or accumulated, throughout the growing season. To compute the growing degree-days for a crop, one has to calculate the mean temperature and then subtract the base temperature from the mean temperature. This gives the number of growing degree-days GDD for each 24-hour period (Edey 1977). The GDD could be calculated from the weather station data, by computing the average temperature over the growing period and multiplying that by the number of days required for either heading or maturity (Edey 1977; Duguid 1990).

One common feature of thermal time assessments is that they all have a base temperature. According to Baker et al. (1986) base temperature determination is important for experiments using thermal units. The most commonly used base temperature is 0°C (Bauer et al 1984). The base temperatures are assumed to remain constant during developmental stages but differ minimally among the cultivars (Slafer and Rawson 1995). The base temperature increases progressively with development, primarily because wheat develops well at low temperature during early developmental periods, whereas relatively warm temperatures are necessary during reproductive phases (Slafer and Rawson 1995). The range of base temperature used in the literature varies between 0 and 10°C.

2.3.2. Photo-thermal unit concept

Nuttonson (1948) suggested a second form of thermal time, where the number of degree-days multiplied by the average day-length was more constant from year to year for a particular location than GDD. These units are known as photo-thermal units (PTU). Light in terms of both quality and quantity is a determining factor for phasic development of heading and maturity phase of wheat crops and their response is greatly influenced by the degree of environmental fluctuations (Patterson 1985). There is evidence that plants continuously monitor the radiation environment and modulate their metabolism and development according to the phytochrome equilibrium established in the plant (Smith 1982). Environmental physiologists and mathematical modelers studied crop development grown under field and controlled conditions to gain further insight into photomorphogenic processes. Many thorough reviews are available on natural radiation spectra but little of this may be directly applied to plant development (Smith 1982). Little information is available on the effect of photo-thermal unit accumulation from solar radiation on spring wheat heading and maturity.

2.3.3. Photon flux concept

Light is a major environmental factor regulating stomatal conductance and photosynthesis. Most plants live in environments with markedly variable light levels. This variation ranges from short-term fluctuations to longer intervals of alternating sun and shade caused by patchy summer cloud cover and photoperiod length (Chazdon and

Pearcy 1991). In a photochemical process such as photosynthesis, the end product (photosynthate production) depends upon the number of quanta of light energy absorbed. The photosynthetic photon flux (also called photosynthetic photon flux density) refers to $\mu\text{moles m}^{-2}\text{sec}^{-1}$ in the useful wavelength region, from 400 to 700 nm (Salisbury and Ross 1992). Not much information is available on the effect of photon flux accumulation from solar radiation on spring wheat heading and maturity.

2.4. Genetic aspects

The replacement of one intact chromosomes from one cultivar with another within the same or different species is known as chromosome substitution. A substitution line results when a chromosome or chromosome pair donated by one source replaces a chromosome or chromosome pair in the recipient. The primary use of substitution lines has been for basic studies on the inheritance of quantitative and qualitative characters (Fehr 1991). The present study deals with reciprocal chromosome substitution lines, produced in spring wheat genetic backgrounds, Rescue or Cadet, which involves transfer by backcrossing. Backcrossing is used to recover the genotype of the recurrent parent except for the chromosome being transferred (Fehr 1991). The studied substitution lines are theoretically genetically identical except for the specific chromosome, in the present study carrying specified *Vrn* genes.

Intervarietal chromosome substitution lines afford the possibility of investigating single chromosomes effects on maturity and other traits on the substituted chromosome (Kuspira and Unrau 1957; Halloran and Boydell 1967). One advantage of chromosome substitution lines is that they only identify additive or epistatic gene action since these lines are homozygous and are different only by a particular substituted chromosome or chromosome pair (Stelmakh 1998). In the study of vernalization requirement and photoperiodic response, chromosome substitution lines of Rescue, Cadet and Cypress was used by Roberts and Larson (1985). Derivates of this work were used in the present study. Chromosome substitution lines are widely used for studying inheritance of quantitative characters of wheat (Law et al. 1976). The development of such lines is time consuming due to labour associated with cytological verifications. Thus microsatellite

markers have been used more recently for quicker verification of complete sets of substitution lines (Korzun et al. 1997).

Near-isogenic lines (NILs; Young et al. 1988) have been developed in many crop species by introgression. They are screened in backcross programs to transfer a gene of interest into desirable cultivars. After each cross, progeny are selected that possess the phenotype expected from the target gene. Pairs of near-isogenic lines have also been developed where one line carries the gene segment of interest in a genetic background, where the remainder of the genome is similar to that of the recurrent cultivated line. Markers can then detect the divergence between the pair of NILs, which are likely be linked to the regions of interest in the genome (Paran et al. 1991).

2.5. Molecular aspects of development

Bossinger et al. (1992) used barley mutants to understand the process of plant development, which lead to mutation-based studies that would culminate in mapping with DNA-based markers. Some of the earlier markers were phenotypic markers that relied on variation in expressed proteins, termed isozymes (Stuber 1992). It is accepted that the gluten proteins, stored in the endosperm of wheat grains, is the main constituent responsible for the viscoelastic properties of wheat flour dough, which makes wheat unique among the cereals (Zhen and Mares 1992). The fraction of gluten proteins soluble in water is called glutenin, while the fraction soluble in 70% alcohol is called gliadin. The high molecular weight glutenin subunits are located on the long arm of group 1 chromosome, while the low molecular weight glutenin subunits are located on the short arm of group 1 chromosome. On the other hand, the gliadin proteins are located on the short arms of group 1 and group 6 chromosome (Zhen and Mares 1992). Wheat gluten protein is studied at the molecular levels due to their abundant transcription levels and highly regulated pattern of expression (Shewry et al. 1997). Protein markers are used in cultivar identification as they differ amongst different genotypes (Cooke 1984). The ability to identify cultivar purity and to select genotypes before seed germination is important in breeding and genetic studies (Chunwongse et al. 1993) and the use of half seed technology to analyse protein in assessing the purity and validation of cultivars

offers a non-destructive sampling method (Lawrence and Shepherd 1980; Zhen and Mares 1992).

Variation in endosperm protein composition (Dexter and Matsuo 1977) and protein components coded by single co-dominant genes have allowed researchers to relate protein components to single genes, merging the work of classical and molecular genetics. Gel electrophoresis of seed proteins has become one of the most important techniques for laboratory cultivar characterization in wheat. Seed storage proteins have been found to be polymorphic with respect to size, charge, or both. These differences in protein profile are characteristic of the wheat genotype (Wrigley and Shepherd 1973). The use of protein electrophoresis to characterize wheat cultivars is favored over the use of morphological characters since they are not affected by environmental conditions (Cooke 1984). Seed proteins have been separated using three main types of gels. These are acidic starch gels, acidic polyacrylamide gels (acid-PAGE), and sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE, Cooke 1984).

With the advent of molecular techniques, genetic difference between individuals can be detected which enables markers to be used for screening traits of interest (i.e. gene(s) of interest). Restriction fragment length polymorphisms (RFLP) are genomic molecular marker (Williams et al. 1990), which detect differences due to nucleotide substitution, insertion, deletion, or rearrangement of the genomic DNA sequence (Chang and Meyerowitz 1991). RFLP's behave in a codominant manner, which allows for determination of the genotype of a plant derived from any two parents (Lander and Botstein 1989).

The genetic map of wheat and its potential in breeding has been advanced by several scientists (Xie et al. 1993; Devos and Gale 1993; Nelson et al. 1995; Williams et al. 1990). Mapping of *Vrn1* genes controlling vernalization requirement, and *Fr1* genes controlling frost tolerance using RFLP markers has also been reported (Galiba et al. 1995). Nelson et al. (1995) reported similar identification of *Vrn1* and *Vrn3* genes in synthetic wheat cross. A population of single chromosome recombinant lines from the crosses between two chromosome substitution lines of Chinese wheat has been developed, and a linkage map of the genes on the long arm of chromosome 5A relative to RFLP marker produced (Kato et al. 1998).

A map of approximately 230 cM in length for chromosome 5A of wheat was constructed, with the gene order from the centromere being *Vrn1-Q-B1* (Sarma et al. 2000). They also reported that a *Vrn1*-adjacent region was located in the central part of the long arm, approximately 90 cM from the centromere. Recently the physical location of the *Vrn1* locus on the long arm of chromosome 5A in wheat has been determined, along with its possible homology with a region on rice chromosome 3 using a common comparative mapping approach (Sarma et al. 1998). Subsequently using deletion mapping, they determined the physical location of the 'earliness per se' gene along with *Vrn1* on chromosome 5A (Sarma et al. 2000).

Molecular markers provide information about genome evolution. Homoeologous loci and co-linearity in chromosome 5 segments of wheat have been identified by several markers (Xie et al. 1993). Some levels of synteny have been observed between genomes of a number of cereals. A comparative mapping of wheat *Vrn-A1* region syntenic with the rice *Hd-6* region on long arm of chromosome 3, has been reported by Kato et al. (1999), which is identified as a QTL controlling heading date through photoperiodic response. Interestingly, *Vrn-A1* was shown to be closely linked to the (*Fr1*) frost resistance gene Sutka et al. (1999). These traits represent some of the characters that need to be brought together in Canadian spring wheat cultivars. Also, RFLP mapping of *Vrn1* on chromosome 5A of wheat, influencing ear emergence time has been reported by Kato et al. (1999), who also reported another putative QTL comparable to *Vrn1*.

Random amplified polymorphic DNA (RAPD) has proven to be an effective molecular marker technique in plant breeding. To be effective in marker-assisted selection, a saturated map with many markers is needed. The advantage of RAPDs is the ability to generate such maps rapidly. RAPD technology uses single short oligonucleotides of synthetic, arbitrary nucleotide sequences to search for variations in genomic DNA by Polymerase chain reaction (PCR) amplification. The amplification reaction, catalyzed by a DNA Polymerase, results in amplification of the products corresponding to DNA between the sequences that bind the primers. PCR involves three basic steps; (1) thermal denaturation of DNA, (2) annealing of oligonucleotide primers to the template DNA Polymerase in the presence of dNTPs. DNA fragments are amplified exponentially for 25 to 45 PCR cycles. The arbitrary primers used for the procedure are

usually 9 to 10 bp in size; they have 50 to 80% C+G content and do not contain palindromic sequences. The number of DNA polymorphisms that are amplified is dependent on the primer and template DNA. RAPD markers may provide a convenient and rapid assessment of the differences in the genetic composition of related individuals (Halward et al. 1992). The polymorphisms can be identified as the presence or absence of particular amplification products, distinguished by their mobility on the ethidium bromide stained agarose gels. The variations in the length of DNA fragments obtained by PCR can be scored in a set of progeny from a genetic cross and treated as a genetic marker. In plants, DNA polymorphisms generated by the RAPD method have been used for assessment of variation (Halward et al. 1992); fingerprinting (Castiglione et al. 1993); cultivar identification (Hu and Quiros 1991); genetic mapping (Williams et al. 1990), and extensive quantitative phylogenetic comparisons of plant genotypes (Halward et al. 1992).

By using arbitrary nucleotide sequence as primers, this technique eliminates the need for target DNA sequence information which is required to build primers for other PCR based systems such as SCAR or microsatellite, and provides an alternative to the labour intensive and lengthy use of southern blotting to detect RFLP's. The markers revealed by these primers behave as dominant traits since a single fragment is either amplified or not amplified at one locus, and they segregate in Mendelian fashion (Williams et al. 1990).

RAPD markers are very simple to use because they do not require DNA sequence information, cloning or synthesis of specific primers. A limitation of RAPD markers is that they are generally dominant; therefore, it is not possible to distinguish quantitatively whether an individual is heterozygous or homozygous on the amplified locus. In general, it is important to distinguish heterozygotes from homozygotes to find tightly linked RAPD markers. If an amplified DNA segment is homozygous, the RAPD fragment will either be present or absent. If it is heterozygous, statistically, the ratio of appearance of the specific amplified band should fit a 2:1 Mendelian segregation ratio. The use of paired dominant markers to detect heterozygotes for a different parental genotype requires twice as many markers as would be needed using co-dominant markers to assess the genotype. However, it has been shown by genetic simulation analysis that dominant markers linked in coupling are as efficient for mapping as co-dominant markers, on a per-

gamete basis (Tingey et al. 1992). Therefore, the dominance drawback of RAPD used as a marker is not a problem. Although it is possible to find markers related to specific phenotypes using RAPD technology, this is not employed in mapping studies. Another potential drawback of using RAPD markers is the non-reproducibility of the results. This is presumably due to low annealing temperature used, by virtue of using short oligonucleotides (decamer primers), and this causes variation in results and makes it often difficult to reproduce the results in different laboratories. This situation can possibly be aggravated in organisms with large genome size (for example, wheat has a genome size of 16 billion bp compared to approximately 70 million bp in *Arabidopsis*) and/or with repetitive DNA sequences.

DNA markers are more reliable as they are phenotypically neutral and are not affected by environmental interactions (Lander and Botstein 1989). Molecular markers are not usually the gene of interest, but are rather linked to the trait of interest (Weeden 1993). A RAPD-based marker linked to the *Bt-10* gene was identified in wheat (Demeke et al. 1996), subsequently used for the development of specific primers to detect the presence of the common bunt *Bt-10* resistance locus (Laroche et al. 2000).

2.6. Bulk segregate analysis (BSA)

Although RAPD analysis can provide an efficient way to detect a particular region in the genome, its application to genetic studies of heterogeneous populations can be limited in cases where a large number of individuals need to be examined. To efficiently find markers related to specific phenotypes, another method called Bulk Segregant Analysis (BSA) has been developed (Michelmore et al. 1991). This is a pooling procedure that greatly increased the efficiency of mapping. BSA involves segregating populations from the extremes of the target trait distribution in the population by artificially separating the DNA samples into two pools. Polymorphic RAPD markers between two bulked samples will be genetically linked to the loci determining the trait. Therefore, BSA can only reveal markers linked to the trait that is used to segregate the population between the two pools. Michelmore et al. (1991) used BSA to identify three RAPD markers linked to major genes using contrasting DNA-bulks composed of F₂ individuals of known genotype for the genes of interest. The bulked segregate method

was also used in a backcross-derived population segregating for a major bean rust resistance gene (Miklas et al. 1993). BSA is recognized as an efficient approach for targeting single gene or multiple loci of the major quantitative trait in a population in which variability is high for all traits.

Amplification Fragment Length Polymorphisms (AFLP) is another technique for DNA fingerprinting which has a wide application for DNA analysis (Vos et al. 1995). Like RAPD, AFLP does not require prior sequence information. However, due to a longer primer sequence containing the restriction site and a few extra nucleotides, the specificity of the PCR is considerably increased, making it much more reproducible. The AFLPs also produce more informative gels due to amplification of several loci at a time, making it much more informative.

Some specific genes involved in determination of flowering (namely *verc* 203 and *verc*17) have been cloned in winter wheat (Chong et al. 1998). These genes are specific to vernalization but little is known about their exact function. It is also not known how these genes are activated during the transition from vegetative growth to flowering. To determine the role of *Vrn* genes in the flowering development, antisense *verc* 203 transformation and expression was carried out which resulted in delayed flowering, indicating possible involvement of these genes in flowering response (Chong et al. 1998). However, in understanding the molecular mechanism of flowering, recent studies have suggested that demethylation of some critical genes may be involved (Brock and Davidson 1994). More recent evidence using anti-sense strategy to interfere with methyl transferase also supports the fact that DNA methylation is an essential component in the process of phase transition and meristem determinancy (Ronemus et al. 1996). Therefore, not only the presence of the gene, but also its methylation state is crucial in determining the phenotype resulting from the presence of a gene.

2.7. Molecular aspects of vernalization

Genetic analyses of naturally occurring early- or late- flowering ecotypes of *Arabidopsis* have been used to identify several genes influencing flowering time. The two major loci accounting for most of the variation in flowering time in *Arabidopsis* are

FRI (FRIGIDA) and FLC (Flowering Locus C) (Aukerman and Amasino 1996). The gene product of FLC has recently been identified and was reported to be involved in the vernalization response. The FLC gene, which encodes a novel MADS-domain transcription factor, was identified through mutant analysis. FLC confers the vernalization requirement, and is also involved in the response to vernalization. Increased expression of FLC leads to delay in flowering, and the down regulation of FLC by cold temperature is proportional to the duration of the cold treatment and the concomitant reduction of flowering time (Sheldon et al. 2000a). In light of these observations, FLC is thought to be the central gene involved in the regulation of flowering through vernalization.

The isolation of the FLC gene and its role in vernalization has provided some understanding of the molecular basis of vernalization. FLC is down-regulated due to both vernalization and demethylation, indicating a role for methylation in vernalization response. However, the mechanism by which FLC is down-regulated by either vernalization or demethylation remains unknown, as does the identity of the genes regulated by FLC. However, there are reports that a number of genes may be involved in the control of flowering by vernalization. Some of these genes are likely to act downstream of FLC (Nelson et al. 2000). Multiple genes that confer insensitivity to vernalization have been mapped in wheat and barley, but their homology to FRI or FLC has yet to be determined (Sheldon et al. 2000b).

Giberellins induce flowering in a number of vernalization requiring plants and hence, have been the focus of studies dealing with biochemical signals involved in vernalization (Bernier et al. 1988, Kinet 1993). In relation to the role of GA's in flower induction, a GA-responsive promoter element has also been identified (Blázquez and Weigel, 2000). Recently it has been reported that in the GA pathway from ent-kaurene (KA) to GA₁₂ are likely to be catalyzed by the cytochrome P450 enzymes which are ent-kaurene oxidase (KO) and ent-kaurenoic acid oxidase (KAO). The isolation of the genes encoding for KAO further reveals that they encode the enzymes for the 3 steps of the GA biosynthesis pathway (Helliwell et al. 2001). However, the exact role of GA's in vernalization and whether FLC contributes to its control has yet to be determined. The two Arabidopsis GA mutants, gall and gai (GA insensitive), exhibit extreme late-

flowering phenotypes only under short-day but not under long-day conditions. Thus the role of GA in vernalization is still speculative since *Arabidopsis* gall mutation did not block the response to vernalization under long-day conditions (Michaels and Amasino 1999). This possibly suggests that GA's may not have a direct role in vernalization, but are required in an alternative pathway which promotes flowering under a non-inductive photoperiod.

In *Thalaspia arvense* cold-induction increased the turnover of kaurenoic acid to 7 β -hydroxy kaurenoic acid by kaurenoic acid hydroxylase (Hazerbroek et al. 1993), but in non-vernalized *T. arvense* plants through the application of DNA methylation agents early flowering could be induced. Based on these results, it has been hypothesized that cold temperature reduces the methylation status of the genes through cold treatment. Vernalization occurs when genes encoding kaurenoic acid hydroxylase are demethylated, thereby allowing its transcription to proceed (Dennis et al. 1996). This is further supported by the early flowering of *Arabidopsis* plants transformed with an anti-sense methyltransferase gene, compared to wild-type ones, in the absence of vernalization (Finnegan et al. 1998).

DNA methylation is an integral part of gene regulatory mechanisms in plants, particularly for the regulation of developmental processes that take place in specific tissues or stages of development. The role of methyltransferases in the establishment and maintenance of methylation patterns is not fully understood, but in future, analyzing the roles of methyltransferases could provide understanding to the ways in which methylation of DNA can regulate gene expression (Finnegan et al. 2000).

The sequence information of the *Arabidopsis* genome should allow the rapid isolation of mutants and genes, which in future could help, in better defining the vernalization process. Similarly, the identification of the genes involved in the biosynthetic pathway of GA biosynthesis should allow scientists to transform plants with both sense- and antisense- orientation of these genes to determine their effect on vernalization.

2.8. Literature cited

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Chapter 3. Effect of chromosome substitution on days to heading and maturity

3.1. Introduction

Spring wheat is normally grown in the short growing seasons of the Canadian Prairies. Hence, successful commercial wheat cultivars must complete their development within a restricted growing period. Canadian spring wheat cultivars exhibit a wide range of maturity (DePauw et al. 1995), as judged by the time required from seeding to heading, and to maturity, when grown in the short summer period. By changing physiological and developmental processes, environmental factors such as day length, temperature, and solar radiation can influence growth patterns of specific cultivars. Variations in rate of development in heading and maturity time for wheat is one of the main components of its adaptation to a wide range of environmental conditions (Wallace and Yan 1998). Inheritance studies of the growth and development of spring wheat (Halloran 1975) have so far failed to produce a very clear picture of the pattern of genetic control underlying these processes. Numerous reports on heading in terms of earliness or lateness suggest a complex genetically controlled system (Crumpacker and Allard 1960; Halloran 1975; Major and Whelan 1985; Goncharov 1998).

Although a number of chromosomes have been identified as affecting the ear emergence time of wheat, the major effects have been found to be associated with the chromosomes of homoeologous group 5, particularly 5A, 5B and 5D (Halloran and Boydell 1967a, b; Zemetra and Morris 1988). These chromosomes have been shown to carry genes that control the response of plants to vernalization treatment (Halloran 1966; Halloran and Boydell 1967a). Vernalization is the acceleration of the plant reproductive development due to exposure to low temperature (Wallace and Yan 1998). The growth habit of wheat is governed by a system of vernalization (*Vrn*) genes, which controls the vernalization response (Pugsley 1972). The *Vrn* genes also determine the division of wheat into spring and winter forms (Islam-Faridi et al. 1996) depending on its developmental response to cold treatment. Several different vernalization genes (*Vrn1* to *Vrn5*) have been identified and localized on group 5 chromosomes of wheat using classical genetics. These are *Vrn1*, *Vrn2*, *Vrn3* (Pugsley 1971, 1972), *Vrn4* (Pugsley

1973), and *Vrn5* (Law 1966). In wheat, the spring growth habit is dominant over winter growth habit, and the presence of a dominant allele of any of the *Vrn* genes effectively inhibits the expression of the winter growth habit (Brulé-Babel and Fowler 1988). On the other hand, winter wheat carry recessive alleles at some *Vrn* loci, and all are sensitive to vernalization (Stelmakh 1990). Vernalization genes *Vrn1*, 3 and 4 are more effective than *Vrn2* or 5 in overcoming the vernalization requirement in wheat (Major and Whelan 1985). To date, the *Vrn* gene composition of most Canada Western Red Spring (CWRS) cultivars is yet to be ascertained.

According to Stelmakh (1987) *Vrn1* on 5A, *Vrn2* on 2B and *Vrn3* on 5D exists in all *Triticum aestivum*, and *Vrn4* on 5B exists in cultivars like Gabo, Shortandinka, Milturum 553, Pirotrix 28, and *Vrn5* on 7B exists in Chinese spring substitution line of Hope. Spring cultivars are the carriers of one or more dominant genes in different combinations, such as the *Vrn4* carriers also possessing *Vrn1*, *Vrn2* and/or *Vrn3* along with other genes. Stelmakh (1998) suggests about one-fourth of the spring cultivars have the monogenic dominant *Vrn1* genotype and up to one-half of them contain *Vrn1* and *Vrn2* in their genotype. The existence of *Vrn3* in combination with different alleles of the other loci was revealed less frequently, and the triple dominant genotype was not found in any of the commercial cultivars (Stelmakh 1998).

Seeding dates on the Canadian prairies vary from location to location and year to year due to variation in weather and soil characteristics (Bootsma and DeJong 1988). Delays in maturity have had, and will continue to have, a very negative impact on Alberta's growers, especially through increased risk of early frost from late harvest. In order to achieve high yield from early- or late- sown spring wheat, farmers should ideally have several genotypes from which to select. These genotypes would differ in their phenological development and/or physiological time to maturity, providing some flexibility in terms of seeding dates.

A better understanding of the effects of multiple seeding dates and the genetics of group 5 chromosome substitution lines in Canada Western Red Spring cultivars may assist in developing early maturing genotypes for the northern regions of the Canadian Prairies. Greater precision in breeding to alter developmental patterns could be achieved if more is known of the separate and combined action of genes influencing

developmental processes (Flood and Halloran 1984) and the environmental control of these effects (Wallace and Yan 1998). These substitution lines could provide useful materials for determining the effect of specific substituted chromosomes carrying *Vrn* gene(s) on heading and maturity in terms of days elapsed (Roberts and Larson 1985; Jedel et al 1986; Wall and Cartwright 1974).

Chromosome substitution lines are widely used for studying inheritance of quantitative characters of wheat (Law et al. 1976). The primary use of substitution lines has been for basic studies on the inheritance of quantitative and qualitative characters (Fehr 1991). The present study employed reciprocal chromosome substitution lines, produced in spring wheat genetic backgrounds, Rescue or Cadet, through backcrossing. The studied substitution lines are genetically identical except for the specific chromosome that is replaced, in the present case, carrying specified *Vrn* genes. Intervarietal chromosome substitution lines offer the possibility of investigating single chromosome effects on maturity and other traits residing on the substituted chromosome (Kuspira and Unrau 1957; Halloran and Boydell 1967a). One advantage of chromosome substitution lines is that they also identify additive or epistatic gene action since these lines are homozygous and are different only for a particular substituted chromosome or chromosome pair (Stelmakh 1998).

The objectives of the present study were: (1) to field characterize heading and maturity for a series of check varieties (Prelude, Park, Katepwa, Columbus) covering the full range of maturity in Canada Western Red Spring wheat (CWRS) in terms of days elapsed under field and controlled environment conditions, (2) to determine the effect of group 5 chromosome substitution on days to heading and days to maturity when planted at different seeding dates (specifically for chromosome 5A - carrying *Vrn1*, 5B - carrying *Vrn4*, and 5D - carrying *Vrn3*, each previously established as substitution lines in a Cadet and a Rescue spring wheat background) under field and controlled environmental conditions; (3) to estimate the effect of the substituted chromosome on days to heading and days to maturity traits through comparisons of the introduced dominant *Vrn* gene(s) in substituted pairs for the specific chromosome that carries the specified *Vrn* genes in both Rescue and Cadet backgrounds, and (4) growth room evaluation of the check

cultivars and substituted lines in days elapsed from seeding to heading and maturity compared to field conditions.

3.2. Materials and Methods

3.2.1. Genetic materials:

Several Canada Western Red Spring wheat (CWRS) cultivars, Cadet, Columbus, Katepwa, Park and Prelude, along with Cadet and Rescue were used as checks, and were chosen to represent the full range of maturity found in current Western Canadian Cultivars. The chromosome substitution lines used were either in Cadet (CR5A, CR5B and CR5D) or Rescue (RC5A, RC5B and RC5D) backgrounds, and were kindly provided by Dr. André Laroche (AAFC, Lethbridge) via Dr. K. G. Briggs (Personal communication). Dr. R. I. Larson had carried out the original development of the substituted lines at Agriculture and Agri-Food Canada Research Centre, Lethbridge Alberta, and the seed stocks were increased. The genetic constitution of the seed stocks used in this study was as follows: Cadet (*Vrn1*), Rescue (*Vrn3*, 4), CR5A (*vrn1*, 3, 4; winter type), CR5B (*Vrn1*, 4), CR5D (*Vrn1*, 3), RC5A (*Vrn1*, 3, 4), RC5B (*Vrn3*), and RC5D (*Vrn4*). In the designation, the first letter indicates the recipient cultivar (C = Cadet; R = Rescue), and the three other letters numbers indicate the source of the specific substituted chromosome.

3.2.2. Field study:

The check cultivars and chromosome substitution lines were grown in experimental field plots at the Edmonton Research Station of the University of Alberta, (53°34'N, 113°25'W) in 1996, 1997 and 1998. The experimental design was a Randomized Complete Block Design with genotypes as main treatments, 2 replicates, in Hill plot arrangement, with hills on 0.5m centers on both directions. Hills sown on the same date were planted in a linear arrangement, separately in each replicate. Five seeds were planted twice weekly in each hill with seeding from mid-April to mid-June, on May 13 - June 12 in 1996, May 5 - June 24 in 1997, and April 20 - June 22 in 1998. The soil type was black chernozemic, and plots were planted into fallow conditions. The plots were hand weeded as necessary, and no herbicides or fungicides were used. Soil nutrient

status in the field was determined by sampling and commercial assay, which indicated no supplemental fertilizer was required in any year.

3.2.3. Phenotypic characterization

The plants were scored for 'days to heading' every second day until 50% of the fertile spikes had emerged from the flag leaf (Elias et al. 1996). For 'days to maturity', the plants were also scored every second day until late-October. The time of loss of all green color from all the fertile spikes was considered to be physiological maturity (Wong and Baker 1986; Hunt and Pararajasingham 1995). The maturity data for 1996 could not be collected due to bird damage prior to ripening. The sowing dates were reported as Julian days (d), which is the consecutive days starting from January 1 (day 1) up to December 31 (day 365) in a non-leap year.

3.2.4. Statistical analysis

The data were analyzed by Proc. means using SAS version 8.1, (year 2000), using days to heading (DH), days to maturity (DM) and days from heading to maturity (DHM) for each year. The mean, standard deviation, and standard error were calculated for each sowing date, and for each quantitative trait (DH, DM and DHM) in CWRS cultivars and chromosome substitution lines. The effects of reciprocal substitution lines for group 5 chromosomes of 5A, 5B, and 5D in either Rescue or Cadet background (introducing dominant genes into recessive) on differences in heading and maturity at multiple planting dates were analyzed by student's t test at 5% significance level ($P=0.05$). These were represented in Tables 3.1 - 3.7 and are different for different years' seeding dates. Also the seeding dates shown in the graphs and in the tables are not the same because they are analyzed differently due to some missing data of maturity. Duncan's multiple range testing was applied on the average air temperature from April to July for 1996, 1997 and 1998, and on the temperature differences between years. The same analysis was used to evaluate differences between the growth chamber data and field data for the check cultivars and substitution lines in relation to lateness for heading and maturity of the growth chamber data from the field for 1997 and 1998. Duncan's multiple range testing was used to detect significant differences in days to maturity between the planting

dates. Analysis of variance was performed to compare the contrasting effect of the substituted line with the original parent in each genetic background. The Statistical model used is: $DH_{ijkl} = \mu + \text{replication (i)} + \text{cultivar (j)} + \text{seeding date (k)} + \text{cultivar} \times \text{seeding date (k,j)} + \text{error}_{ijkl}$, where $i = 1, 2$; $j = 1, 2 \dots 4$; and $k = 1, 2, \dots 18$ (as this were not the same each year).

3.2.5. Growth-chamber studies:

The chromosome 5D substituted lines and the check cultivars were also grown in growth chambers, and days to heading and days to maturity were determined under controlled environmental conditions. These genotypes were used since the 5D substitution lines were used for characterization of the segregating F_2 population. Since only the crosses segregating for *Vrn3* were examined, the other substitution lines were not grown in the growth chambers. Ten seeds for each genotype were grown individually in 12.5 cm plastic pots containing MetroMix® in growth chambers (15°C day/10°C night, 16 hr photoperiod). The pots were arranged randomly in blocks of 5, with 2 replications for each cultivar and substitution line, and were periodically moved within treatments to reduce the environmental variation within the growth chamber and to achieve uniform light intensity. The light intensity at plant level was $350 \mu\text{mol m}^{-2}\text{s}^{-1}$ from high output fluorescent lights. The main shoots were staked for stability and tagged for days to heading and maturity, and the plants were watered daily and fertilized twice weekly with Peters 20-20-20™ containing micronutrients. The data analysis was carried out as described previously for field data with 5 plants considered as a replication units.

3.3. Results

3.3.1. Field study:

This work was conducted for 3 years (1996, 1997 and 1998) at the Edmonton Research Station, University of Alberta, and the data were collected for days to heading (DH) and days to maturity (DM), from which days from heading to maturity (DHM) was

also calculated. As indicated in 1996, maturity data were not available due to extensive bird damage in all seeding dates (1996 figures shown in appendix Fig. 7.1, 7.2).

3.3.1.1. Effects of seeding date on days to heading and maturity:

3.3.1.2. Check cultivars:

In all years, delayed seeding reduced the number of days required for heading in all check cultivars (Fig. 3.1A, B) until early June dates, after which they tended to level off. The general trend in check cultivars was Prelude, Park, Katepwa and Columbus in order of increasing days required for heading. Another trend observed was the progressively less days required for heading by the cultivars in later sowing dates. There was no significant effect of sowing date on maturity in terms of days required for check cultivars in 1997 (Fig. 3.2A). In both 1997 and 1998 Prelude matured first followed by Park, Katepwa and Columbus (Fig. 3.2A, B).

Prelude was found to be the earliest cultivar for heading and maturity in all years, which took 53 days for heading (Fig. 3.1B) and 110 days for maturity (Fig. 3.2B) when sown on April 20, 1998. Columbus, on the other hand, required 64 days for heading (Fig. 3.1B) and 117 days to mature (Fig. 3.2B) when sown on April 20, 1998. Park and Katepwa were the intermediate cultivars in all years, both in terms of heading and maturity (Fig. 3.1A, 3.2A).

3.3.1.3. Substitution lines:

In the case of the chromosome substitution lines, chromosome 5B from Rescue (carrying *Vrn4*) into Cadet (CR5B) in presence of *Vrn1* was found to have the greatest effect in reducing time to heading (Fig. 3.3A, B) and time to maturity (Fig. 3.4A, B). When sown on April 20, 1998, CR5B (*Vrn1*, 4) required 61 days for heading (Fig. 3.3B) and 111 days for maturity (Fig. 3.4B), followed by 5A from Cadet into Rescue background (RC5A) (carrying *Vrn1*, 3, 4), which required 58 days for heading (Fig. 3.5A) and 111 days for maturity (Fig. 3.5B). The substitution lines containing chromosome 5D from Cadet into Rescue background (RC5D), (carrying *Vrn4*) was

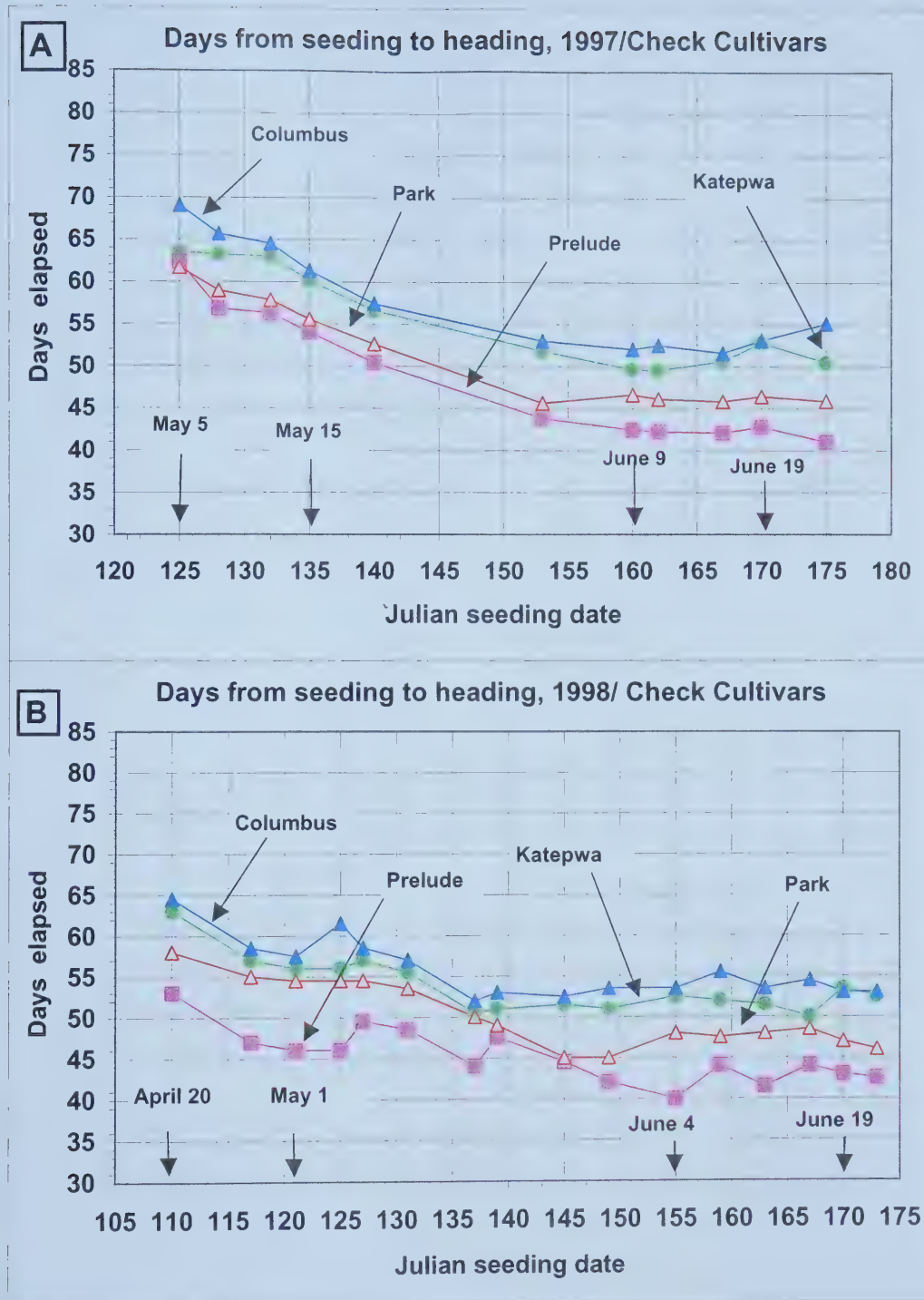


Fig. 3.1. The effect of seeding dates on days elapsed from seeding to heading in 1997 (A) and 1998 (B) in check cultivars at Edmonton Research Station.

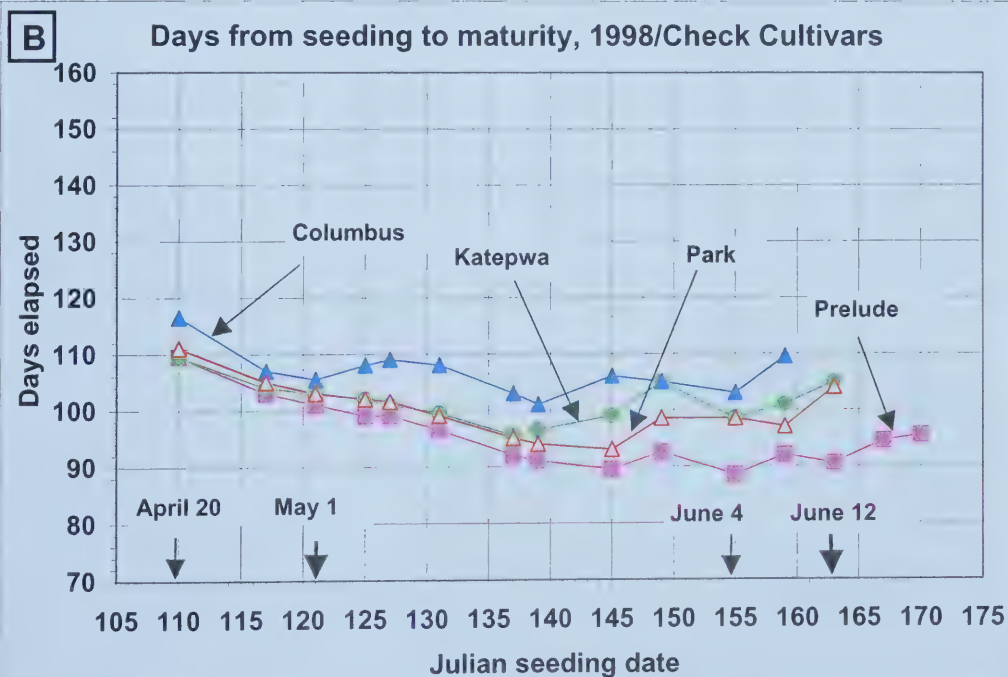
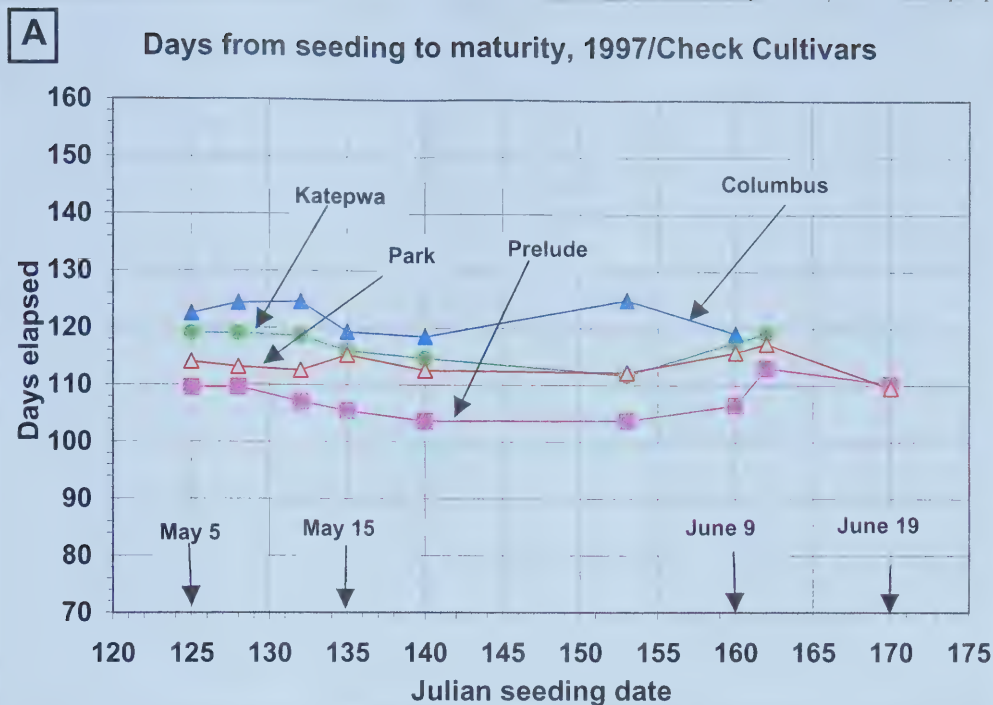


Fig. 3.2. The effect of seeding dates on days elapsed from seeding to maturity in 1997 (A) and 1998 (B) in check cultivars at Edmonton Research Station.

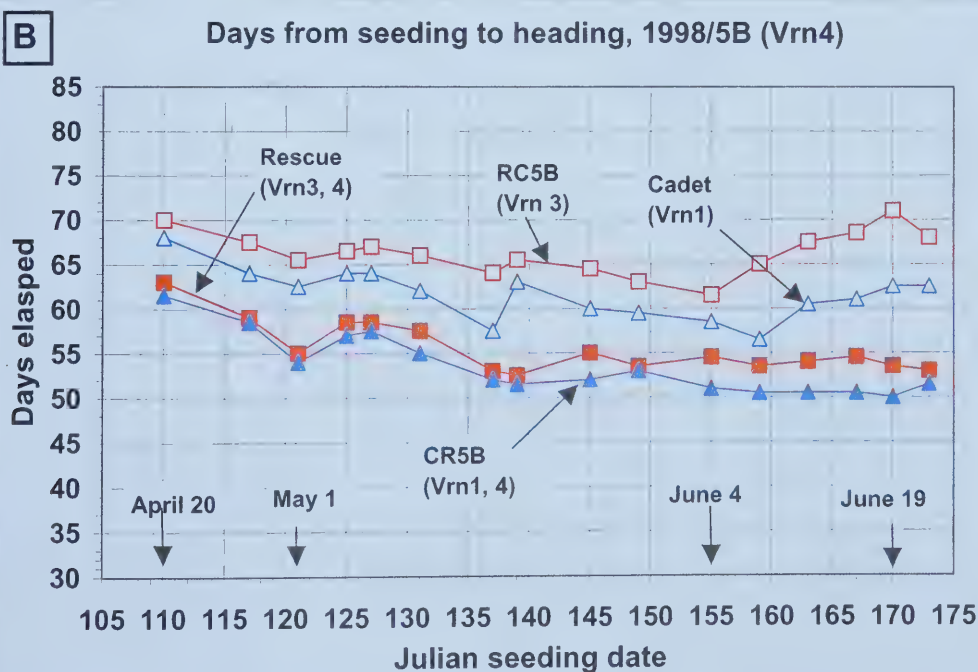
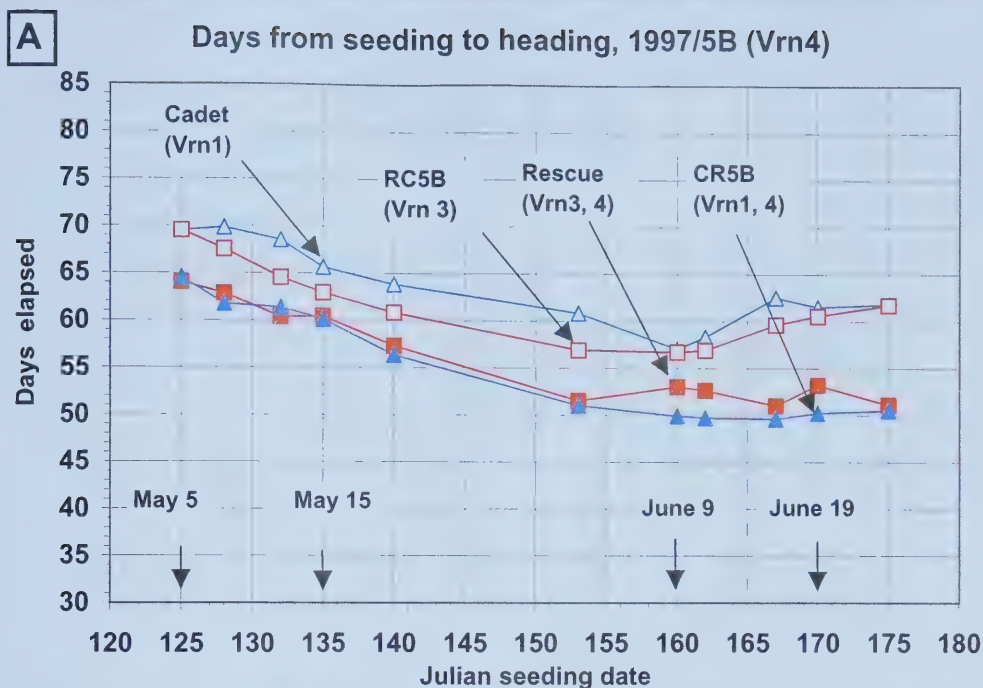


Fig. 3.3. The effect of seeding dates on days elapsed from seeding to heading in 1997 (A) and 1998 (B) in chromosome 5B substitution lines and their recipient parents at Edmonton Research Station.

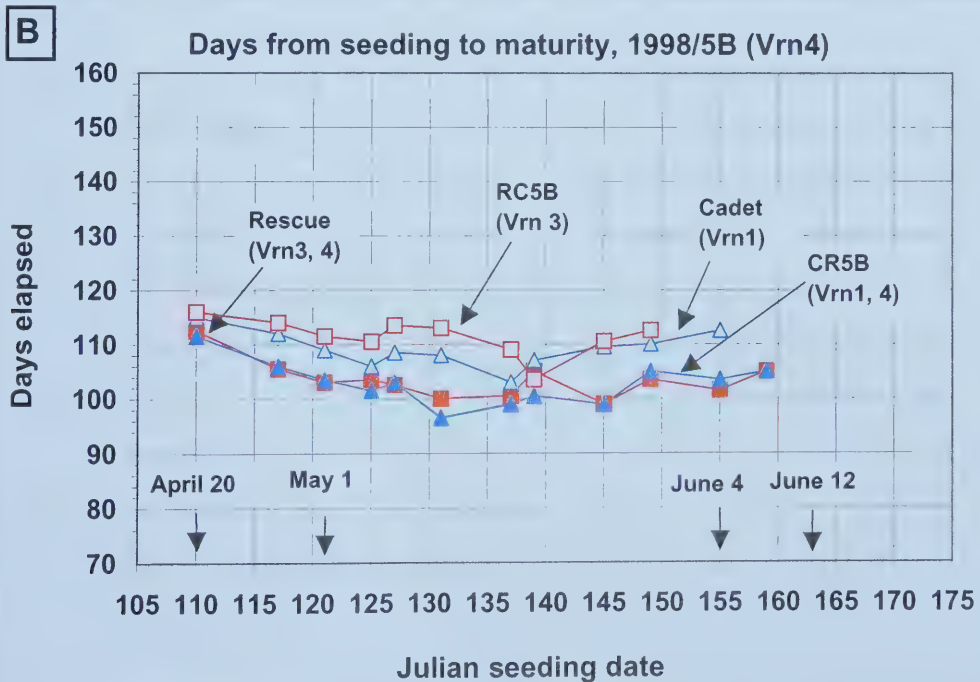
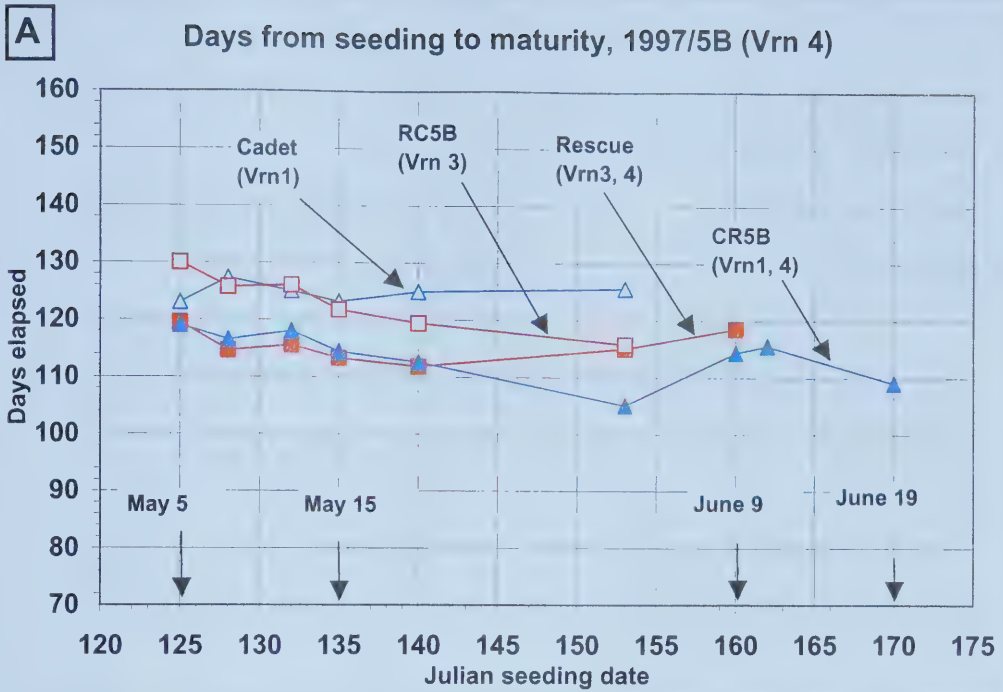


Fig. 3.4. The effect of seeding dates on days elapsed from seeding to maturity in 1997 (A) and 1998 (B) in chromosome 5B substitution lines and their recipient parents at Edmonton Research Station.

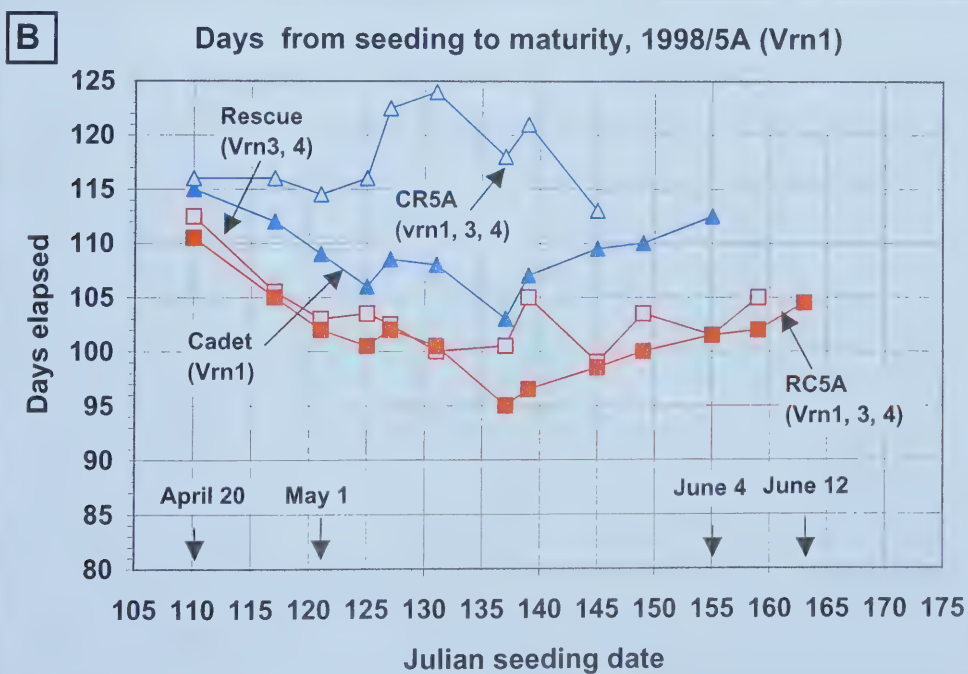
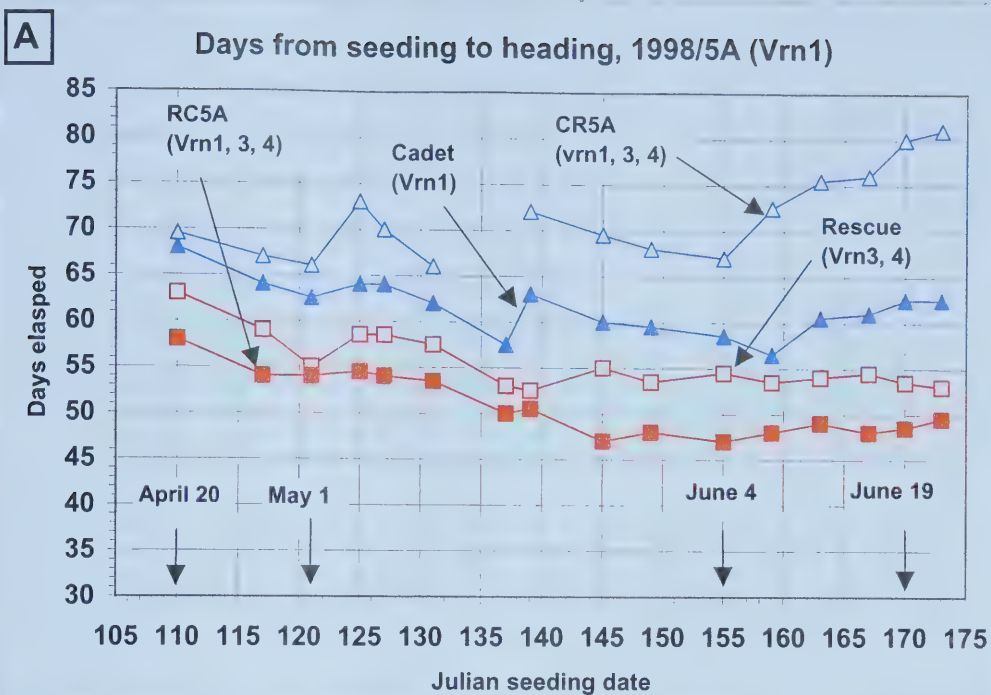


Fig. 3.5. The effect of seeding dates on days elapsed from seeding to heading (A) and seeding to maturity (B) in 1998 in chromosome 5A substitution lines and their recipient parents at Edmonton Research Station.

found to head later, which required 64 days (Fig. 3.6 A) and earlier 111 days to maturity (Fig. 3.6B) than the Rescue (*Vrn3*, 4) when sown on April 20, 1998. In 1997, no consistent trend was observed in maturity in terms of days among substituted lines carrying *Vrn1* (Fig. 3.7A) and *Vrn3* (Fig. 3.7B). CR5A was unable to produce heads in 1996 (see appendix, Fig. 7.1B), and in 1997 it headed (Fig. 7.3A) and matured (Fig. 3.7A) in the first two seeding dates, but in 1998 it headed in most seeding dates (Fig. 3.5A) and matured in some seeding dates (Fig. 5.3B). The computed variables days from heading to maturity provided inconsistent results without any clear trend (Fig. 3.8A, B).

3.3.2. Effect of introducing group 5 chromosomes:

The quantitative differences between the recipient parents of each chromosome substitution are shown in tabular form for the years 1996, 1997 and 1998 (Table 3.1 to 3.7), reported here as the effect of substituting a chromosome that carries an additional dominant *Vrn* gene. It was observed that most of these effects were in the direction of earlier maturity.

The most pronounced genetic effect was observed due to the introduction of chromosome 5B from Rescue (*Vrn4*) into Cadet background (CR5B) in terms of reduced number of days required for heading in 1996 (e.g. 12.0 days at May 25, Table 3.1). The reciprocal chromosome substitution into Rescue background (RC5B) was not as effective in reducing the number of days for heading (Table 3.1). The introduction of the 5B chromosome of Rescue (*Vrn4*) into Cadet background (CR5B) showed a maximum reduction of 9.8 days for heading than its reciprocal, Rescue background (RC5B) 5.4 days, when sown on June 2, 1997 (Table 3.2). The most prominent genetic effect observed in 1998 heading was also due to the introduction of the 5B chromosome of Cadet (*vrn4*) into Rescue background (RC5B) resulting in a reduction of 13.0 days (May 19) for heading compared to the introduction of the 5B chromosome from Rescue (*Vrn4*) into Cadet background (CR5B), which resulted in a significant reduction of 11.5 days in (May 19) heading days compared to Cadet (Table 3.3). The largest genetic effect was observed due to the substitution of the 5B chromosome of Rescue (*Vrn4*) into Cadet background (CR5B) that resulted in the highest significant reduction of 20.5 days in maturity compared to Cadet when planted on June 2, 1997 (Table 3.4). On the other

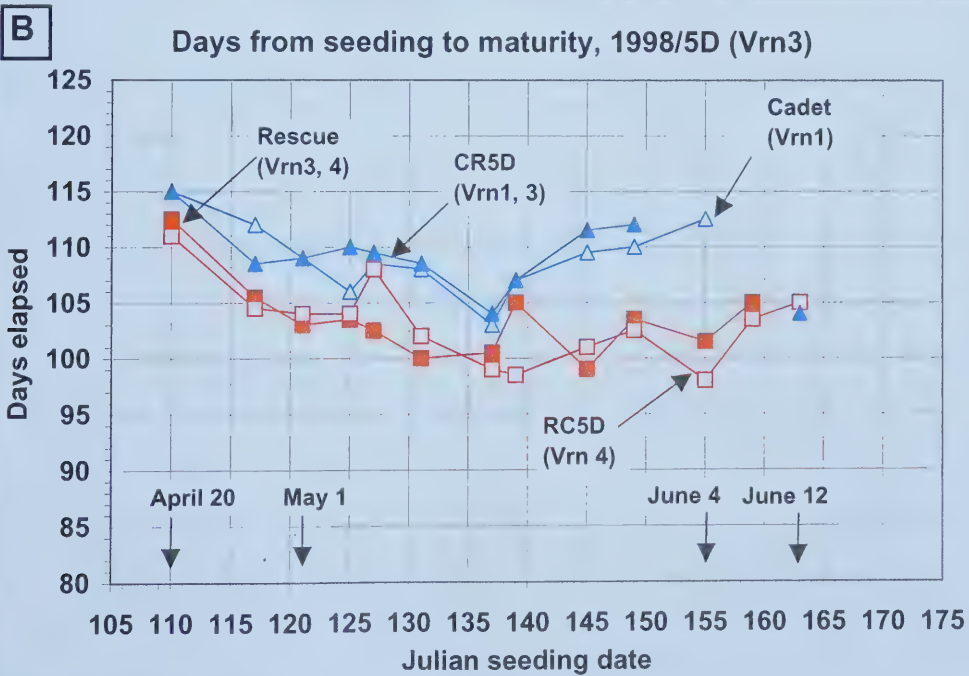
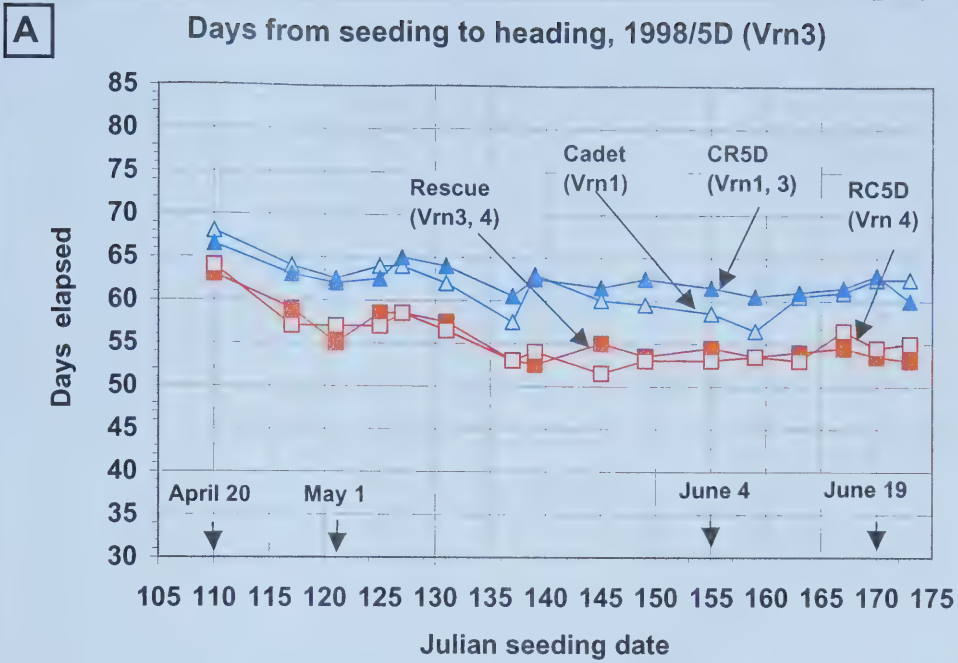


Fig. 3.6. The effect of seeding dates on days elapsed from seeding to heading (A) and seeding to maturity (B) in 1998 in chromosome 5D substitution lines and their recipient parents at Edmonton Research Station.

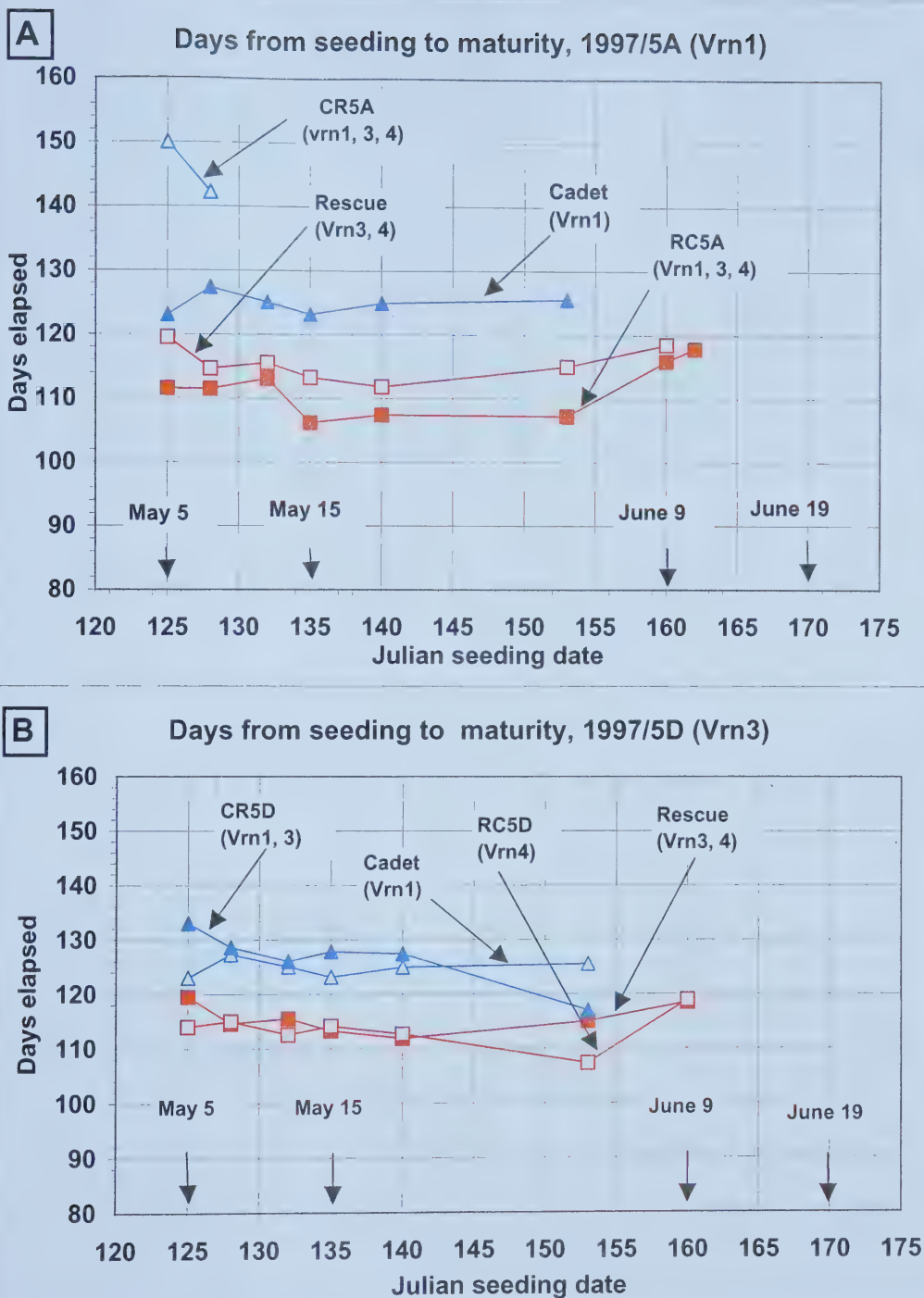


Fig. 3.7. The effect of seeding dates on days elapsed from seeding to maturity in 5A (A) and 5D (B) chromosome substitution lines and their recipient parents in 1997 at Edmonton Research Station.

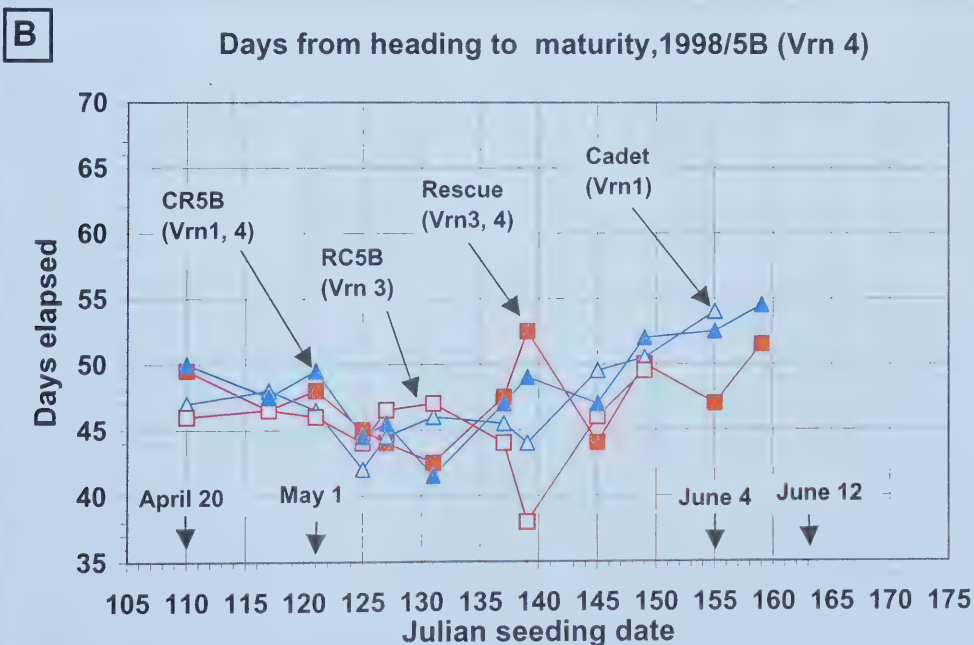
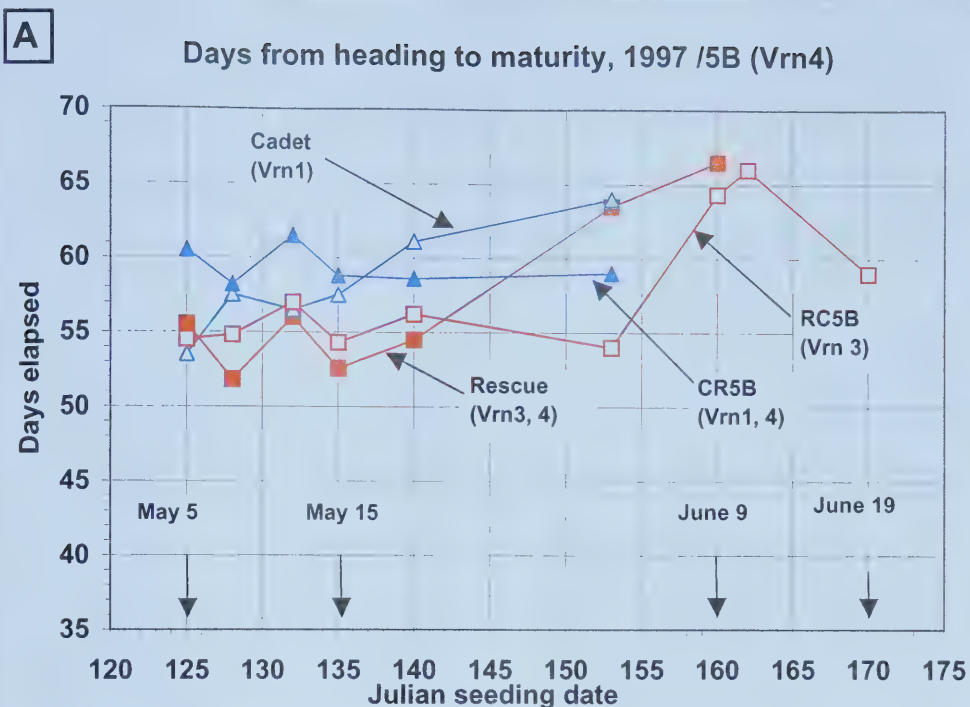


Fig. 3.8. The effect of seeding dates on days elapsed from heading to maturity in 1997 (A) and 1998 (B) in chromosome 5B substitution lines and their recipient parents at Edmonton Research Station.

Table 3.1. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds, on number of days required for heading in multiple planting dates at Edmonton Research Station in 1996. (* indicates significant differences, $P < 0.05$)

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date:						
May 13	- [†]	-4.5	-7.5*	-3.5	-2.0	-0.5
May 16	-	-2.0	-8.0	-3.5	1.0	1.0
May 19	-	-7.0*	-9.0*	-1.0	0.0	0.0
May 22	-	-0.5	-6.0	-6.5*	3.5	1.0
May 25	-	-5.0	-12.0*	-1.5	-1.0	1.5
May 29	-	-5.5	-7.0*	-2.5	0.0	1.0
May 31	-	-8.0*	-5.5*	-3.0	-0.5	1.0
June 3	-	-6.0	-2.0	-3.0	0.0	0.0
June 6	-	-5.0	-3.0	-3.0	0.5	-0.5
June 12	-	-4.5*	-6.5	-3.5	-0.5	0.5

[†] No comparison possible due to absence of heading in CR5A.

Table 3.2. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds, on number of days required for heading in multiple planting dates at Edmonton Research Station in 1997. (* indicates significant differences, $P < 0.05$)

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date:						
May 5	-15.5	-2.0	-5.0	-5.5*	2.0	-3.0
May 8	-18.0	-1.1	-8.1*	-4.7*	-0.2	0.4
May 12	- [†]	0.6	-7.1*	-4.1	0.0	-1.6
May 15	-	-3.7*	-5.5*	-2.5*	0.0	0.6
May 20	-	-3.7*	-7.4*	-3.5*	-0.6	0.5
June 2	-	-5.2*	-9.8*	-5.4*	-1.9*	-1.8*
June 9	-	-7.5*	-7.1*	-3.7*	-0.5	-0.7

[†] No comparison possible due to absence of heading in CR5A.

Table 3.3. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds, on number of days required for heading in multiple planting dates at Edmonton Research Station in 1998. (* indicates significant differences, $P < 0.05$)

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-1.5	-5.0*	-6.5	-7.0	-1.5	-1.0
April 27	-3.0	-5.0*	-5.5	-8.5*	-1.0	2.0
May 1	-3.5	-1.0	-8.5*	-10.5*	-0.5	-2.0
May 5	-9.0	-4.0	-7.0	-8.0	-1.5	1.5
May 7	-6.0	-4.5	-6.5*	-8.5*	1.0	0
May 11	-4.0	-4.0*	-7.0	-8.5*	2.0	1.0
May 17	-	-3.0	-5.5*	-11.0	3.0	0
May 19	-9.0*	-2.0	-11.5*	-13.0*	-0.5	-1.5
May 20	0.5	-3.0	0.5	-10.0	5.0	0
May 25	-9.5	-8.0	-8.0	-9.5	1.5	3.5
May 29	-8.5*	-5.5	-6.5*	-9.5*	3.0	0.5
June 4	-8.5	-7.5*	-7.5	-7.0*	3.0	1.5
June 8	-16.0*	-5.5*	-6.0*	-11.5*	4.0*	0

[†] No comparison possible due to absence of heading in CR5A.

Table 3.4. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds on number of days required for maturity in multiple planting dates at Edmonton Research Station in 1997. (* indicates significant differences, $P < 0.05$)

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-27.0	-8.0	-4.0	-10.5	10.0	5.5
May 8	-14.9*	-3.2*	-10.8*	-11.1*	1.3	-0.4
May 12	- [†]	-2.5	-7.0	-10.5	1.0	3.0
May 15	-	-7.1*	-8.7*	-8.4*	4.7*	-0.8
May 20	-	-4.4	-12.3*	-7.6*	2.5	-0.8
June 2	-	-7.7*	-20.5*	-0.7	-8.5	7.7*
June 9	-	-2.6	-	-	-	-0.3

[†] No comparison possible due to absence of heading in CR5A.

Table 3.5. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds, on number of days required for maturity in multiple planting dates at Edmonton Research Station in 1998. (* indicates significant differences, $P < 0.05$)

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-1.0	-2.0	-3.5	-3.5	0.0	1.5
April 27	-4.0	-0.5	-6.0*	-8.5	-3.5*	1.0
May 1	-5.5*	-1.0	-5.5*	-8.5	0.0	-1.0
May 5	-10.0	-3.0	-4.5*	-7.0	4.0	-0.5
May 7	-14.0	-0.5	-5.5*	-11.0*	1.0	-5.5
May 11	-16.0	0.5	-11.5	-13.0*	0.5	-2.0
May 17	-15.0	-5.5	-4.0	-8.5*	1.0	1.5
May 19	-14.0	-8.5	-6.5	1.5	0.0	6.5
May 20	- [†]	-3.0	-6.5*	-9.0	-3.0	-0.5
May 25	-3.5	-0.5	-10.5*	-11.5	2.0	-2.0
May 29	-	-3.5	-5.0	-9.0	2.0	1.0
June 4	-	0.0	-9.0*	-	-	3.5
June 8	-	-3.0	-	-	-	1.5

[†] No comparison possible due to absence of heading in CR5A.

Table 3.6. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds, on number of days required from heading to maturity in multiple planting dates at Edmonton Research Station in 1997. (* indicates significant differences, $P < 0.05$)

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-11.5	-4.5	1.0	-5.0	8.0	8.5
May 8	2.2	-2.1	-2.7	-6.4*	1.5	-0.8
May 12	- [†]	-4.0	0.5	-5.5	1.0	6.0
May 15	-	-3.1	-3.2*	-6.2*	4.7*	-1.7
May 20	-	-0.7	-4.8*	-4.1	3.1	-1.3
June 2	-	-2.7	-10.0*	4.5	-6.0	9.5*
June 9	-	3.8	-	-	-	1.0

[†] No comparison possible due to absence of heading in CR5A.

Table 3.7. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue backgrounds, on number of days required from heading to maturity in multiple planting dates at Edmonton Research Station in 1998. (* indicates significant differences, $P < 0.05$)

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	0.5	3.0	3.0	3.5	1.5	2.5
April 27	-1.0	4.5	-0.5	0.0	-2.5	-1.0
May 1	-2.0	0.0	3.0	2.0	0.5	1.0
May 5	-1.0	1.0	2.5	1.0	5.5*	-2.0
May 7	-13.5	4.0	1.0	-2.5	0.0	-5.5
May 11	- [†]	4.5	-4.5	-4.5	-1.5	-3.0
May 17	-	-2.5	1.5	3.5	-2.0	1.5
May 19	-6.0	-6.5	5.0	14.5*	0.5	8.0
May 20	4.0	3.0	-8.5	1.5	-10.5	-1.5
May 25	-	5.5	3.0	1.0	5.5	2.5
May 29	4.5	7.5	-2.5*	-2.0	0.5	-5.5
June 4	-	2.0	1.5	0.5	-1.0	0.5
June 8	-	7.5	-1.5	-	-	2.0

[†] No comparison possible due to absence of heading in CR5A.

hand, the introduction of the 5B chromosomes of Cadet (*Vrn4*) into Rescue parental line (RC5B) resulted in a significant reduction of 11.1 days in maturity compared to Rescue when planted on May 8, 1997 (Table 3. 4).

In Rescue background, the greatest genetic effect was observed when 5B chromosomes of Cadet (*vrn4*) were introduced into Rescue background (RC5B) resulting in a maximum reduction of 13.0 days (May 11) for maturity compared to the introduction of 5B chromosome from Rescue (*Vrn4*) into Cadet background (CR5B), which resulted in a significant reduction of 11.5 days in (May 11) maturity compared to Cadet in 1998 (Table 3.5). In terms of days from heading to maturity (DHM) in 1997, a greater genetic effect was observed when 5B chromosomes of Rescue (*Vrn4*) was introduced into Cadet background (CR5B) with a maximum significant reduction in 10.0 days (June 2, 1997) for DHM compared to Cadet (Table 3.6) than the introduction of 5B chromosome from Cadet (*vrn4*) into Rescue background (RC5B) which resulted in a significant reduction of 6.4 days in (May 8) DHM compared to Rescue (Table 3.6). The introduction of the Rescue 5A (*Vrn1*) chromosome into Cadet background was unable to produce any significant differences in DHM in the first two seeding dates and other differences were unable to be calculated, as this line did not head in 1997 (Table 3.6). The presence of the 5D chromosome of Cadet (*vrn3*) into Rescue background (RC5D) resulted in a significant increase of 9.5 days in DHM compared to Rescue when planted on June 2, 1997, whereas the introduction of the 5D chromosome of Rescue (*Vrn3*) into Cadet background (CR5D) also resulted in a significant increase of 4.7 days in DHM when planted on May 15, 1997 (Table 3.6).

In terms of DHM in 1998, the most pronounced genetic effect was also observed in 5B chromosomes of Cadet (*vrn4*) when introduced into Rescue background (RC5B) with a maximum reduction in 14.5 days (May 19) for DHM than the introduction of 5B chromosome from Rescue (*Vrn4*) into Cadet background (CR5B) which resulted in a significant reduction of 2.5 days in (May 29, 1998) maturity compared to Cadet (Table 3.7). The introduction of the Cadet 5A (*Vrn1*) chromosome into Rescue background (RC5A) or Rescue 5A (*Vrn1*) chromosome into Cadet background did not produce any significant results in DHM differences, whereas the introduction of the 5D chromosome

from Rescue (*Vm3*) into Cadet background (CR5D) resulted in a significant reduction of 5.5 days in DHM when planted on May 5, 1998 (Table 3.7).

In all 3 years, the greatest number of significant substitution effects was found with 5B in both Cadet and Rescue background, and these were of the largest magnitude, resulting in up to 13 days earlier maturity from Cadet 5B into Rescue background (RC5B) (Table 3.5) in 1998. Very small effects for 5A substitution lines, 5.5 days were observed from Rescue 5A when introduced into Cadet background in the third seeding date of 1998, and may be attributed to earliness in maturity in CR5A (Table 3.5) whereas from Rescue 5D when introduced into Cadet background, was 3.5 days earlier in Cadet background in the second seeding date in 1998 (Table 3.5). Notwithstanding the significant effects of 5B (Table 3.5) substitution, and other significant effects in Table 3.4, 3.5, 3.6 and 3.7, these results are characterized by a fairly high level of inconsistency from year to year. This two-replicate experiment had a relatively high coefficient of variation associated with its protocol, so that many large effects observed were not necessarily significant.

3.3.3. Growth chamber study:

Under controlled environment conditions, Prelude required the least number of days for heading (53), followed by Park (73), Katepwa (89) and Columbus (98), (Table 3.8a). The substituted line RC5D and its recipient parent (Rescue) behaved similarly in terms of days to heading, as did CR5D and Cadet (Table 3.8a). Similar trends were observed in days to maturity (Table 3.8a). The controlled environment study paralleled the work completed in the field for 3 years although the duration of the phases measured in days were longer compared to the field study (Table 3.8b).

3.3.4. Interaction between seeding dates and cultivars:

Analysis of variance showed contrasting effects between the substituted lines with the recipient parents Rescue and Cadet. The most significant results were obtained in the 5B substituted lines for heading. In 1996, there were significant differences in heading in the chromosome substitution line CR5B due to different seeding dates (Table 3.9). The

5A substitution effect could not be determined due to the lack of heading for CR5A in 1996, and heading occurred in 1997 for only two dates, whereas the 5D substitution did not produce any significant effect on heading in different seeding dates in either Cadet or Rescue backgrounds. In 1997, RC5A and RC5D substitution lines heading showed differences due to different seeding dates. In 1998, CR5A and CR5B showed significant difference between the seeding date and the substitution lines. In CR5A, such effect was observed for maturity only in 1998 (Table 3.9).

Duncan's multiple range test showed that the average temperature from April to July of 1996, 1997 and 1998 was 10°C, 11°C and 14°C respectively. The average temperature in 1998 April was 8°C. There was no statistical difference observed between temperatures in 1996 and 1997, but in 1998 the temperature was higher 14°C, (see results in Table 7.3 at Appendix).

It was observed that the average days to maturity differed significantly between planting dates. There were usually three to six groupings from the Duncan's multiple range tests. An example of such analysis is shown in the appendix for 1996 (Table 7.9).

3.4. Discussion

The check cultivars exhibited a wide range in days to heading and maturity over all years studied. The relative ranking as judged by the time taken from seeding to heading were consistent and delayed seeding dates produced early heading, which ranged from 42-68 days for the check cultivars. The *Vrn* gene composition in the check cultivars is not available in the published literature but the ranking of the cultivars in respect to earliness agrees with a published report (DePauw et al. 1995). The series of 5B substitution lines studied did cover a similar maturity range to that found in the Canadian check cultivars selected. Therefore, the Cadet/Rescue chromosome substitution system was potentially suitable to explain the full range of heading and maturity differences in relation to days required for the phenological development.

Table 3.8a. Days elapsed from seeding to heading and maturity in spring wheat genotypes grown in growth chambers at 15°C/16hr days, 10°C nights.

Cultivar	Days to heading (\pm S.E.)	Days to maturity (\pm S.E.)
Prelude	52.7 (1.11)	139.0 (4.83)
Park	72.7 (2.22)	178.3 (3.35)
Rescue	73.4 (1.44)	168.6 (4.16)
RC5D	75.9 (1.55)	163.8 (5.14)
Katepwa	88.9 (1.79)	183.4 (5.24)
CR5D	96.3 (2.23)	184.0 (2.75)
Cadet	97.9 (1.82)	181.4 (1.61)
Columbus	98.3 (2.20)	188.7 (1.61)

Table 3.8b. Days from seeding to heading (DH) and seeding to maturity (DM) in cultivars and substituted lines of wheat grown in 1996, 1997 and 1998 at Edmonton Research Station.

	1996	1997		1998	
Cultivar	DH (\pm S.E.)	DH (\pm S.E.)	DM (\pm S.E.)	DH (\pm S.E.)	DM (\pm S.E.)
Prelude	49.5 (0.64)	48.1 (0.91)	106.7 (0.66)	44.8 (0.73)	94.2 (1.06)
Park	55.5 (0.37)	51.3 (0.57)	113.9 (0.43)	49.6 (0.83)	98.8 (1.07)
Rescue	58.5 (0.46)	57.8 (0.48)	114.4 (0.66)	54.4 (0.82)	101.9 (1.02)
RC5D	58.0 (0.47)	57.8 (0.44)	113.5 (0.76)	54.0 (0.78)	101.8 (0.95)
Katepwa	56.5 (0.72)	56.5 (0.55)	116.2 (0.49)	53.1 (0.69)	100.0 (0.97)
CR5D	62.5 (0.77)	66.0 (0.50)	127.2 (0.90)	60.4 (0.96)	107.0 (1.28)
Cadet	63.0 (0.86)	66.2 (0.45)	125.0 (0.71)	60.0 (0.90)	107.7 (0.93)
Columbus	58.5 (0.58)	60.3 (0.55)	121.5 (0.70)	55.0 (0.75)	105.5 (1.01)
CR5A	-	85.8 (1.70)	143.0 (4.77)	65.0 (1.79)	113.4 (2.33)
RC5A	54.0 (0.83)	52.7 (0.65)	110.6 (0.71)	49.6 (0.87)	100.0 (0.96)
CR5B	56.5 (0.55)	54.0 (0.51)	114.2 (0.55)	53.0 (0.75)	101.3 (1.05)
RC5B	62.0 (0.55)	63.3 (0.47)	122.2 (1.05)	64.3 (0.82)	110.0 (1.15)

Table 3.9. Analysis of variance for days to heading (DH) and days to maturity (DM) for 1996, 1997 and 1998 showing the effect of seeding date on chromosome substitution lines. (* indicate significant, and ns indicate non-significant difference at 5% level, – indicates missing data).

			1996	1997	1998
Cadet/ CR5A	DH	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	ns	*
	DM	Seeding date	-	ns	*
		Cultivar	-	*	*
		S.date x Cultv.	-	*	*
Rescue/ RC5A	DH	Seeding date	*	*	ns
		Cultivar	*	*	ns
		S.date x Cultv.	ns	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	ns	ns
Cadet/ CR5B	DH	Seeding date	*	*	*
		Cultivar	*	*	*
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	*	ns
Rescue/ RC5B	DH	Seeding date	*	*	*
		Cultivar	*	*	*
		S.date x Cultv.	ns	*	ns
	DM	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	ns	ns
Cadet/ CR5D	DH	Seeding date	*	*	*
		Cultivar	ns	ns	ns
		S.date x Cultv.	ns	ns	ns
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns
Rescue/ RC5D	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	ns
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns

The chromosome 5B substitution lines may play an important role through affecting a faster development rate in wheat in the absence of vernalization and photoperiod influence, as has been suggested by Flood and Halloran (1983). Evidence on the possible influence of a higher temperature requiring process for reproductive development in long day plants has been reported in wheat (Gott 1957), which agrees with our present findings of later seeding resulting in early heading. Thus, in wheat different temperature requirements in different seeding dates between substitution lines to determine the rate of development is evident.

A complexity exists with the 5A comparisons in the Cadet background, as the line CR5A is a putative winter type, and is believed to carry all the recessive *vrn* genes (*vrn1*, *vrn2*, *vrn3*, *vrn4*, *vrn5*). This line was able to form heads in the first two seeding dates in 1997, and the 9 seeding dates in 1998 (Fig. 5A), which suggest that it is possibly not a true winter type. CR5A was entered in the trial in the belief that it was a true winter type, and would require vernalization in order to head. On the basis of three years of data, and if it is a true winter type (multiple recessive *vrn* genes), its vernalization requirement is obviously minimal, since it was able to form heads in 1997 and 1998. It is possible that CR5A only headed where it had been vernalized naturally in the field. This is similar to the reported late heading of Cadet-Rescue 5A substitution lines in the field (Roberts and McDonald 1984).

Several wheat phenology models have endorsed a broad optimum temperature for vernalization. Porter et al. (1987) and Kirby (1992) assumed temperatures between 3-10°C were equally optimal for vernalization of winter wheat, while temperature either lower than 3°C or higher than 10°C, were usually less effective. In contrasts with 0-3°C usually used to vernalize germinating seeds in many studies, it has been reported that 15°C (Purvis 1961), 17°C (Wallace and Yan 1998), even up to 19°C (Rawson et al. 1998) could be rated as a vernalizing temperature. The average temperature from April to July in 1998 was 14°C, whereas in 1996 and 1997 it was 10°C and 11°C respectively. Thus, it is possible that CR5A genotypes were vernalized *in situ*, resulting in its heading in 1997 and 1998.

The present finding suggests that there could be some unexplainable effects of vernalization, and is consistent with the published findings that vernalization could occur at higher temperatures. Nevertheless, variability in earliness exists within the sets of chromosome substitution lines that have the same *Vrn* gene constitution and such differences could be due to the differences in sensitivity to growing temperatures and/or light intensity (Stelmakh 1998). Alternately, such an effect could be due to the different genetic backgrounds used in the study.

The introduction of the homeologous group 5 chromosomes (5B) into their recipient parents Cadet or Rescue resulted in a reduction of the time required for heading in all the years studied (Table 3.1-3.3, 3.9). These chromosomes carry vernalization (*Vrn*) genes, which control the sensitivity of plant response to vernalization treatment (Halloran 1966; Halloran and Boyde 1967a). In the present study, the substitution of chromosome 5B Rescue into Cadet background (CR5B) or Cadet 5B into Rescue background (RC5B) resulted in the most pronounced effect on earliness by reducing the time necessary for heading and maturity compared to the other two substituted chromosomes, 5A and 5D (Tables 3.1-3.5, 3.9). This trend was consistently observed over all years of the study. Thus, the present results are in agreement with the previous finding that these chromosomes of group 5 influences ear emergence time (Halloran and Boyde 1967a, b; Zemetra and Morris 1988) and maturity (Driscoll and Jensen 1964). Driscoll and Jensen (1964) suggested the presence of genes for earliness in 5B, and a gene for lateness in 5D, which could explain the earliness of 5B over 5D. Chromosome 5A was reported to have a gene for lateness or an ineffective (hemizygous) one for earliness (Driscoll and Jensen 1964), which could also account for delay of heading in RC5A and CR5A compared to RC5B and CR5B, as observed in the present study (Tables 3.1-3.5). However, in 5B, the observed result indicated significant interaction in terms of heading in all three years studied (Table 3.9). In the present case, the effects of the seeding date on substitution lines shows that there are differences between the studied lines. These substitution lines are contrastingly different from Cadet and Rescue cultivars. The D genome of hexaploid wheat is considered to have greatly increased the adaptive range of wheat (Zohary et al. 1969), but there are also claims that this contribution is not immediately apparent. It has been shown that chromosome 5D of

Hope in Chinese Spring (Halloran 1975) and Thatcher in Chinese Spring (Flood and Halloran 1983), can either hasten or slow development respectively, in the absence of vernalization and photoperiod influences.

The number of days elapsed for heading has always been the reported variable for cereals (Stelmakh 1993, 1998; Major and Whelan 1985; Flood and Halloran 1984; Wallace and Yan 1998), but data on days to maturity, and heading to maturity calculations for spring wheat are not widely available in the published literature. The results obtained from heading over the 3-year study period were consistent, but the results of maturity and days from heading to maturity did not show any consistent trend. This two-replicates experiment in the field for all years had a relatively higher coefficient of variation associated with this protocol, so many large effects were not significant.

No evidence was found from the field trials that day length changes from delayed seeding date caused any consistent change in maturity among the substitution lines. This result is consistent with the possibility that the day length requirements for heading are fully met in all seeding dates at Edmonton for all Canada Western Spring wheat cultivars and the chromosome substitution lines, by the long photoperiod present from the early to late seeding date in this location. In general terms, such a hypothesis suggests that day length itself, and the genetic complement for photoperiod genes, may not be a factor in determining differences in heading and maturity for the chromosome substitution lines in this particular location. For a long day crop such as wheat, there is a minimum (or maximum) optimal photoperiod (MOP) which could be defined as the shortest photoperiod such that increasing the photoperiod beyond this value does not increase the rate of development (Shaykewich 1995). This could possibly explain why we did not observe any effect of photoperiod in the present study, but the effects of factors like growing degree-days (GDD), photo-thermal units (PTU) or photon flux units (PF) that vary throughout the season, cannot be ruled out.

Although the prevailing temperature varied due to climatic conditions in the three years during the study, the day-length period did not change for a particular seeding date over the three-year period. Therefore temperature seems to be the driving force in the observed differences between the years, rather than the photoperiod. The environmental

variables such as temperature and photoperiod interacting with *Vrn* and other genes on the substituted chromosome could play an important role in determining the maturity differences in Canadian Spring wheat grown at this northern latitude.

The chromosomal effects (of 5A, 5B and 5D) were similar each year and no crossover occurred, and a similar response pattern was observed in the check cultivars. According to Stelmakh (1998), 20-25% of the variation in the heading date of wheat may be attributed to genetic differences in the photoperiod gene system (photoperiodic response) but in the current study the effect of the photoperiod or light intensity did not seem to have any effect on all seeding dates or may be confounded with other environmental factors (see chapter 4).

According to Stelmakh (1993), in near isogenic lines with Triple-Dirk (TD) background, the presence of *Vrn1* resulted in a requirement of 37-39 days to head, compared to *Vrn3* requiring 46-47 days, indicating major differences in response to heading in these two lines. However, in the present study, no major difference in heading was observed. For instance, Cadet (containing *Vrn1*) required between 68-70 days for heading, compared to RC5B (containing *Vrn3*) requiring 60-70 days. It should be noted that the relative number of days required for heading in the current study was considerably greater than that reported (Stelmakh 1993), which could be due to different growing conditions, or due to differences in the genetic backgrounds. The same reasons may also account for differences observed between the *Vrn* genes.

In the present study, the lines containing Rescue 5B chromosomes were observed to be the earliest ones to head and mature over all years studied (Fig. 3.3, 3.4) and substitution that with chromosome 5B from either Cadet or Rescue resulted in the greatest effect on days to heading and maturity. These effects could be due to the presence of *Vrn4* on this chromosome. The presence of *Vrn4* has been shown to result in earlier heading and maturity in other wheat lines (Driscoll and Jensen, 1964). In both Rescue and Cadet background the presence of *Vrn4* had the largest effect in reducing the time to heading and maturity, followed by *Vrn1*, and finally, *Vrn3* (Tables 3.1-3.5). In 1997, due to different seeding dates, no consistent trend was observed in maturity in terms of days among substituted lines carrying *Vrn1* (Fig. 3.7A) and *Vrn3* (Fig. 3.7B). In

1997 and 1998, the substitution lines of *Vrn4* in both backgrounds matured early in late seeding dates (Fig. 3.4A, B). Therefore, due to different seeding dates, *Vrn4* showed a more pronounced effect towards early heading and maturity in comparison to the *Vrn1* and *Vrn3* substitution lines.

The effect of substituting chromosome Rescue 5D (*Vrn3*) was found to be mostly small to insignificant. The 5D substitution effects were not large enough to show significant differences in maturity, as these lines responded in ways similar to their recipient parents (Fig. 3.6B, 3.7B). The present study also revealed the fact that effect of the *Vrn1* gene is more pronounced than *Vrn3*, which is in agreement with previous findings (Maystrenko, 1980). The relative effect of *Vrn3* is also influenced by prevailing temperature (Stelmakh, 1998), and this could account for the variability in the results observed in 1997 and in 1998. Another possible explanation could be the presence of a high level of environmental variation, observed in the present study, which is associated with hill plot data, possibly masking the 5D (*Vrn3*) effect. Thus, the results show that the effects of the introduced dominant gene (*Vrn3*) in both backgrounds is characterized by a fairly high level of inconsistency from year to year (Table 3.1-3.7), and the frequent large effects which are seen in these data, were not statistically significant. A larger number of replicates or Randomized block design might be helpful in reducing the variability in the data sets, but such an approach would involve a substantial increase in labour and cost.

In the growth chamber, the cultivars and the chromosome substitution lines required a considerably longer time to head and mature, as compared to the field. The cooler temperature in the growth chamber (15°C day/10°C night 16 hour photoperiod), compared to prevailing summer temperatures (approximately 10-22°C in 1996 to 1998) in the field, may have contributed to the developmental delay in heading and maturity. Development of cereals is certainly affected by temperature, photoperiod and the amount of the radiation (Thorne et al. 1968). Also, changes in day length (photoperiod), temperature, and variation in light intensity, a common occurrence under field conditions, did not occur in the controlled environment. Therefore, the plants were possibly lacking the environmental cues for a developmental switch, since there was only minor environmental variation under the controlled conditions in the growth chamber over the growing period.

There was little scope for studying the influence of intensity of radiation, as the maximum intensity attainable in the growth chambers are far less than that achieved outdoors at any time during the natural growing season (Thorne et al. 1968). The specific gene effects on developmental rate and their influence on heading and maturity are also probably difficult to explain in the checks since their *Vrn* genetic composition is not known. Despite this effect, the growth chamber ranking for earliness remained the same as in the field, for check cultivars and for the chromosome substitution lines. Duncan's multiple range test showed significant differences existed between the growth chamber which took a longer time than usual for heading, when compared to field-grown cultivars. Some heat unit variables (GDD, PTU or PF) were also analyzed for the lateness in the growth chamber and in the field, and these results are discussed in the Chapter 4.

The analysis of variance suggested that group 5 substituted lines of Rescue 5B (for heading and maturity) and seeding date interactions were significant for all years compared to Cadet 5A and Rescue 5D. These differences between the substitution lines were confirmed by other reports in the literature.

Differences in the rate of development (heading and maturity time) for wheat are the main components contributing to their adaptation to a wide range of environmental conditions. The *Vrn* genes (growth habit), the photoperiod genes and the 'earliness' gene all contribute to these differences (Goncharov 1998) that further interact with several environmental components. The *Vrn* gene effects can contribute up to 75% of differences in the total length of the wheat life cycle (Stelmakh 1998).

Overall, it was observed that due to later sowing dates the cultivars and the chromosome substitution lines tended to head earlier, compared to earlier sowing dates. The quantitative effect of introducing a chromosome that carries an additional dominant *Vrn* gene fulfills the expected results by showing the greatest effects in the direction of early maturity. This trend of earliness was consistent in terms of heading for all three years in the field study, but the results for maturity showed variation from year to year. While considering days from heading to maturity, this variation was even of larger magnitude, possibly resulting from the additive effect of the experimental error from both days to heading and days to maturity. This resulted in the DHM trends being statistically

insignificant and inconsistent in many cases. Notwithstanding this, the substitution of chromosome Rescue 5B (carrying *Vrn4*) resulted in reduced number of days to heading and maturity as compared to Cadet 5A (carrying *Vrn1*) and Rescue 5D (carrying *Vrn3*).

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Chapter 4. Effect of environmental factors on heading and maturity

4.1. Introduction

The majority of the agricultural areas of Canada are characterized by a very short growing season so early maturity is a desired trait in spring wheat. Successful commercial wheat cultivars must complete their development within this restricted growing period. Variation in environmental factors through seasonal changes can affect the crops' phenological development, and these environmental variables and influences of photoperiod (Gott 1961), vernalization (Flood and Halloran 1986) and temperature (Aitken 1966; Syme 1968) determine the heading and maturity in spring wheat (Jordon 1993).

For many years, it was believed that the period of heading and grain maturity is of similar duration among varieties but that considerable time differences exist between seeding and heading (Chun, 1993). More recently, significant temporal variations have been shown for all stages of wheat development such as time to emergence, basic vegetative phase, and the duration of all stages between spike initiation and stem elongation, and grain filling and maturation (Slafer and Rawson 1994).

The two most important abiotic factors influencing plant development from seeding to maturity are temperature and photoperiod (minimal optimal photoperiod and sensitivity) (Slafer and Rawson 1994). Most Canada Western Red Spring (CWRS) cultivars are photosensitive. Thus, temperature, both high and low, may be the most important factor for many spring wheat varieties. Exposure to low temperature is essential in many types of wheat to decrease the vegetative phase (Jedel et al. 1986). This is under the control of vernalization (*Vrn*) genes (Major and Whelan 1985).

The genetics and the physiology, through which temperature and photoperiod jointly influence the time a cultivar needs to complete development to its days to heading and days to flowering, are not well understood (Wallace and Yan 1998). Temperature affects the rate of plant growth. Each plant has its own specific optimum temperature for growth, and a temperature range, over which it thrives. The heat unit required is

expressed as degrees of temperature. The concept of growing degree-days assumes that plant growth is related directly to the average daily temperature. The degree-days for each day are added together, or accumulated, throughout the growing season (Edey 1977). To compute the growing degree-days for a crop on a particular day, one has to calculate the daily mean temperature and then subtract the base temperature from the mean temperature of the crop in question. This number gives the number of growing degree-days (GDD) for a 24-hour period (Edey 1977). Therefore, the heat units or GDD is a combination of calendar time and daily temperature towards measuring plant development (Klepper et al. 1998). Degree-days permit comparisons of differences in growing potential. The accumulation of GDD is one method of predicting crop maturity, rather than simply counting the days elapsed.

Nuttonson (1948) suggested the number of degree-days multiplied by the average day-length was more constant from year to year for a particular location than GDD, resulting in the calculation of another form of thermal time known as photothermal units (PTU). There is evidence that plants continuously monitor the radiation environment and modulate their metabolism and development according to the phytochrome equilibrium established in the plant (Smith 1982). Environmental physiologists and mathematical modelers have studied crop development grown under field and controlled conditions to gain further insight into photomorphogenic processes (Holmes and Smith 1977). The total number of photosynthetically active light quanta accumulated throughout the growing season can be calculated by summing the total radiation over the growing period. The influence of the photon flux (PF) accumulation on wheat development using the natural variation of solar radiation from day to day has the advantage that the responses observed can be used more readily to infer behavior for crop manipulation under conditions similar to the field.

The genetic control of growth and developmental phases of wheat is a complex phenomenon determined by several factors. Five different vernalization genes (*Vrn1* to *Vrn5*) have been identified and three have been localized on group 5 chromosomes of wheat using classical genetics. The major genes controlling variation in the ear emergence time in hexaploid wheat *Vrn1*, *Vrn3*, *Vrn4* are now well characterized. These

homoeoallelic genes for vernalization requirement (*Vrn1*, *Vrn4*, *Vrn3*) are located on the long arms of chromosomes 5A, 5B, and 5D respectively (Law et al 1976; Maystrenko 1980; Stelmakh 1993; Stelmakh 1998). Vernalization genes *Vrn 1*, *Vrn 3* and *Vrn 4* are the more effective than *Vrn2* or 5 in fulfilling the vernalization requirement in wheat (Major and Whelan 1985). Along with the *Vrn* genes there are photoperiod genes as *Ppd1* controls the photoperiodic responses along with its homoeolog *Ppd2* and *Ppd3* genes located on the wheat chromosomes 2D, 2B and 2A respectively (Keim et al. 1973; Klaimi and Qualset 1973). Different stages of development can be exposed to very different photoperiodic regimes (Slafer and Rawson 1995). An understanding of the substituted chromosomes carrying the genes controlling early maturity is needed if they are to be used in a breeding program in a systematic and effective manner.

Seeding dates on Canadian prairies vary from location to location and year to year, as a result of variation in weather and soil characteristics (Bootsma and DeJong 1988). Early maturity is an important characteristic for wheat producers at northern latitudes, where growing conditions are characterized by short seasons, cool temperatures and potential for early frost.

A better understanding of the effects of seeding dates and group 5 chromosome substitution lines in Canadian cultivars, may assist in developing early maturing genotypes for the northern regions of the Canadian Prairies. Precise genetic control of flowering time is of importance to most wheat breeders, and a better understanding of the environmental factors involved can lead to improved cultivar adaptation (Syme 1973). Information on heat units and their effects on agronomic traits are of great value to wheat breeders and for agriculture. Thus, spring wheat substituted chromosome lines can be used to breed commercial cultivars for the prairie regions, through exploitation of a simple inherited character for better adaptation of the crop plant to its agricultural environments.

The present experiments were conducted to obtain a better understanding of the developmental components of time to heading and maturity in check cultivars with respect to heat accumulation in terms of GDD, PTU, and PF. Also, group 5 chromosome

substitution lines of spring wheat in either Rescue or Cadet background were used to see the effects of the substituted chromosome (5A, 5B, and 5D) in terms of the heat accumulated for time to heading and maturity.

The objectives of the present study were: (1) field characterization of the heat unit accumulation as growing degree days (GDD), photothermal units (PTU) and accumulated photon flux (PF) on heading and maturity traits for a series of check varieties (Prelude, Park, Katepwa and Columbus) of unknown *Vrn* gene composition covering the full range of maturity in CWRS wheat, (2) to determine the requirement of GDD, PTU and PF on group 5 chromosome substitution sets of 5A carrying different combinations of *Vrn1*, *Vrn3* and *Vrn4* in Cadet or Rescue background for heading, days and maturity traits in the field, (3) to study the influence of seeding date on heat units required for heading and for maturity traits, calculated in terms of heat unit accumulation as growing degree-days (GDD), photothermal units (PTU) and accumulated photon flux (PF) from seeding to heading and maturity.

4.2. Materials and Methods

4.2.1. Genetic materials:

CWRS cultivars (Columbus, Katepwa, Park, Prelude) along with Cadet and Rescue were used as checks, and were chosen to represent the full range of maturity found in wheat cultivars currently grown in Western Canada. The chromosome substitution lines used were either in Cadet (CR5A, CR5B and CR5D) or Rescue (RC5A, RC5B and RC5D) backgrounds, and were kindly provided by Dr. Andre Laroche (AAFC, Lethbridge, via Dr. K. G. Briggs, (Personal communication). The original development of the substituted lines had been carried out in Agricultural and Agri-Food Canada Research Station at Lethbridge, Alberta, and the seed stocks were increased. Two crosses segregating for *Vrn3* (5D) in either Rescue (Rescue, *Vrn3*, 4 x RC5D, *Vrn4*) or Cadet (CR5D, *Vrn1*, 3 x Cadet, *Vrn1*) background were also made at the University of Alberta, Edmonton. The genetic constitution of the seed stocks used in this study were as

follows: Cadet (*Vrn1*); Rescue (*Vrn3*, 4); CR5A (*vrn* winter type); CR5B (*Vrn1*, 4); CR5D (*Vrn* 1, 3); RC5A (*Vrn1*, 3, 4); RC5B (*Vrn3*); and RC5D (*Vrn4*). In the designation, the first letter indicates the recipient cultivar (C= Cadet; R= Rescue) and the three other letters indicate the source of the specific substituted chromosome.

4.2.2. Field design:

The plants were grown in experimental field plots of the Research Station at the University of Alberta in Edmonton (53°34'N, 113°25'W) in the summers of 1996, 1997 and 1998. The experimental design was a Randomized Complete Block Design with 2 replicates, in Hill plot arrangement, with hills of 0.5m centers on both directions. Hill plots sown on the same date were planted in a linear arrangement, separately in each replicate. Five seeds were planted twice weekly in each hill with seeding between mid-April to mid-June with the seeding dates being May 13 - June 12 in 1996, May 5 - June 24 in 1997, and April 20 - June 22 in 1998, with the genotypes as the main treatment. The soil type of the area was black chernozemic, and plots were planted into fallow conditions. The plots were hand weeded as necessary, and no chemicals, herbicides or fungicides were used on the plots. Soil nutrients in the field were estimated by sampling and commercial assay, and no supplemental fertilizer for wheat was required in any year.

4.2.3. Phenotypic characterization:

The plants were scored for days to heading every other day until 50% of the fertile spikes had emerged from the flag leaf (Elias et al. 1996). For days to maturity, the plants were also scored every other day until late-October and the loss of all green color from all the fertile spikes was considered to be the stage of full maturity (Wong and Baker 1986; Hunt and Pararajasingham 1995).

4.2.4. Statistical analysis:

The data were analyzed by Proc. means using SAS version 8.1, (year 2000), using days to heading (DH), days to maturity (DM) and days from heading to maturity (DHM) for each year. The mean, standard deviation and standard error at each sowing date and for each quantitative trait (DH, DM) in CWRS cultivars and chromosome substitution lines

were calculated. The effects of reciprocal substitution lines for group 5 chromosome of 5A, 5B, and 5D in either Rescue or Cadet background (introducing dominant genes into recessive) on differences in heading and maturity at multiple planting dates were analyzed by student's t test at 0.05% significance level. These were represented in the Tables 4.1-4.21 and are different for different years seeding dates. Also the seeding dates shown in the graphs and in the tables are not the same because they were analyzed differently due to some missing data of maturity. The maturity data for 1996 could not be collected due to extensive bird damage in the plots prior to ripening. The DHM data provided an unacceptable high coefficient of variation because of additive error from the two component variables (DH and DM), and no further analysis was conducted on the variables on DHM data. Studies of this variable would require more replicates than available in this trial. Correlation matrix was calculated by SAS (Proc. Corr) to determine the relationship among the three variables (GDD, PTU and PF) used in the experiment. The analysis of variance was performed on the seeding dates, substitution lines and the interaction between seeding date x substitution lines at each variable to look at the significant differences between the substituted lines and their recipient parents. The Statistical model used is: $DH_{ijkl} = \mu + \text{replication (i)} + \text{cultivar (j)} + \text{seeding date (k)} + \text{cultivar} \times \text{seeding date (k}_j) + \beta \text{ (GDD or PTU or PF)} + \text{error}_{ijkl}$, where $i = 1, 2$; $j = 1, 2 \dots 4$; and $k = 1, 2 \dots 18$ (as this were not the same each year). The sowing dates are reported as Julian days (d), which is the consecutive days staring from January 1 (day 1) up to December 31 (day 365) in a non-leap year.

4.2.5. Environmental data:

Weather data (maximum, minimum and mean air temperature, photoperiod and solar radiation) for each year was collected from the Edmonton Research Station Weather facility by using a LI 190 SB Quantum Sensor data recorder. The sensor was calibrated to 6.25 microamps/1000 $\mu\text{mole m}^{-2} \text{ s}^{-1}$. Converted to millivolts, this would be 3.775 mV/ $\mu\text{mole m}^{-2} \text{ s}^{-1}$. This output units represents total flux in $\text{mmole m}^2/\text{day}$. The prevailing weather conditions (hourly temperature, photoperiod, and light intensity) were obtained from the Research Station for calculating various parameters used in the study.

4.2.6. Growing Degree-Days:

The Growing Degree Days (GDD) statistics was calculated by computing the average temperature over the growing period and multiplying that by the number of days required for either heading or maturity. The base temperature was taken as 0°C. The time and duration of each developmental stage and developmental period were expressed in GDD. Accumulated GDD for a particular stage or period was calculated by summation of daily degree-days (T_n). Daily degree-days were calculated as:

$$T_n = (T_{\max} + T_{\min}) / 2 - T_b,$$

where, T_{\max} and T_{\min} refers to the maximum and minimum daily air temperature, respectively, accumulated during a 24 hr interval from midnight to midnight, and T_b is the base temperature below which no wheat development occurs.

In the literature both 0° (Gallager 1979; Baker et al. 1980; 1986) and 5° C (Yasuda and Shimoyama 1965) have been used as base temperatures. In this study, a base temperature of 0° and 5° C initially was used in analyzing the data but since no difference was observed in the results except the scale in graph shifting slightly, 0° was finally used following the procedures of Gallager (1979) and Baker et al. (1980, 1986). The GDD for a single day can be represented according to Edey (1977) by the following equation:

$$K_1 = (T_m - T_0),$$

where, T_m =Mean temperature, T_0 =Base temperature, and K_1 =Daily degree-days.

4.2.7. Photo-thermal units:

For calculating the Photo-thermal units (PTU), the number of days required for either heading or maturity was multiplied by the average temperature over the period (as in GDD), which was in turn was multiplied by the average hours of sunlight over the same period. Photo-thermal units (PTU) are the products of degree-days (T_n) and daylight hours (Nuttonson, 1948) given by the formula:

$$K_2 = L (T_m - T_0),$$

where, L =Photoperiod, T_m =Mean temperature, T_0 =Base temperature, and K_2 =Photo-thermal units.

4.2.8. Photon flux:

The photon Flux (PF) was calculated by summing the total daily incident radiation over the growing period required for either heading or maturity at each seeding date.

4.2.9. Growth-chamber studies:

The 5D substituted lines and the check cultivars were also grown in growth chambers and days to heading and days to maturity were determined under conditions of 15°C day/10°C night, 16 hr photoperiod. These genotypes were used since the 5D substitution lines were used for characterization of the segregating F_2 population. Since only the crosses segregating for *Vrn3* were examined, the other substitution lines were not grown in the growth rooms. Ten seeds from each plant were grown individually in 12.5 cm plastic pots containing MetroMix[®]. The pots were arranged randomly in blocks of 5, with 2 replications on the bench for each cultivar and substitution line, and were periodically moved within treatments to reduce the environmental variation within the growth chamber and to achieve uniform light intensity. The light intensity at plant level was 350 $\mu\text{mol m}^{-2}\text{s}^{-1}$ from high output fluorescent lights. The main shoots were staked for stability and tagged for days to heading and maturity, and the plants were watered daily and fertilized twice weekly with Peters 20-20-20[™] containing micronutrients. The data analysis was carried out as described previously for field data with the average of 5 plants considered as a replication units.

4.3. Results

4.3.1. Seeding date effects on heading and maturity

4.3.1.1. Check cultivars

In 1997, there was a reduction in GDD requirement for heading in the later seeding date (Fig. 4.1A), but in 1998 no apparent effect on GDD requirement for heading was observed related to different sowing dates for all check cultivars due to high level of variation (Fig. 4.1B). No major effect of sowing date on GDD requirement for maturity was observed in 1997 and 1998 (Fig. 4.2A, B). Prelude required less GDD to mature early followed by Park, Katepwa and Columbus in 1997 (Fig. 4.2A). In 1998, cultivars sown early required more GDD to mature, which gradually decreased. In the early sowing date of April 20, (1998), Prelude required 1840 GDD to mature, followed by Park which required 1850 GDD to mature whereas Katepwa required 1820 GDD and Columbus required 1950 GDD accumulation to mature (Fig. 4.2B). The graphs for both years showed variability in terms of GDD requirement for maturity. Due to the large variability in the maturity data, the results for GDD, PTU and PF requirement in check cultivars and the substitution lines are not discussed any further. Similarly, due to the absence of maturity data in 1996, no maturity calculations could be made and the graphs for 1996 are thus shown in the appendix (Fig. 7.1, 7.2, 7.4 and 7.5).

When results are expressed on a PTU basis, the trends observed were similar to GDD requirement for heading, showing early heading at later sowing dates. A reduced level of photothermal units required for heading was apparent in 1997 (Fig. 4.3A) but the PTU did not show any effect in 1998 (Fig. 4.3B). The effect of later sowing showed a consistently lower PF requirement for heading (Fig. 4.4A, B) during the study period. The ranking of the check cultivars remained the same in each year for heading and maturity. When sown on June 22 of 1998, Prelude required 1350 mmol/m², Park 1500 mmol/m², Katepwa 1750 mmol/m² and Columbus required 1750 mmol/m² PF for heading (Fig. 4.4B).

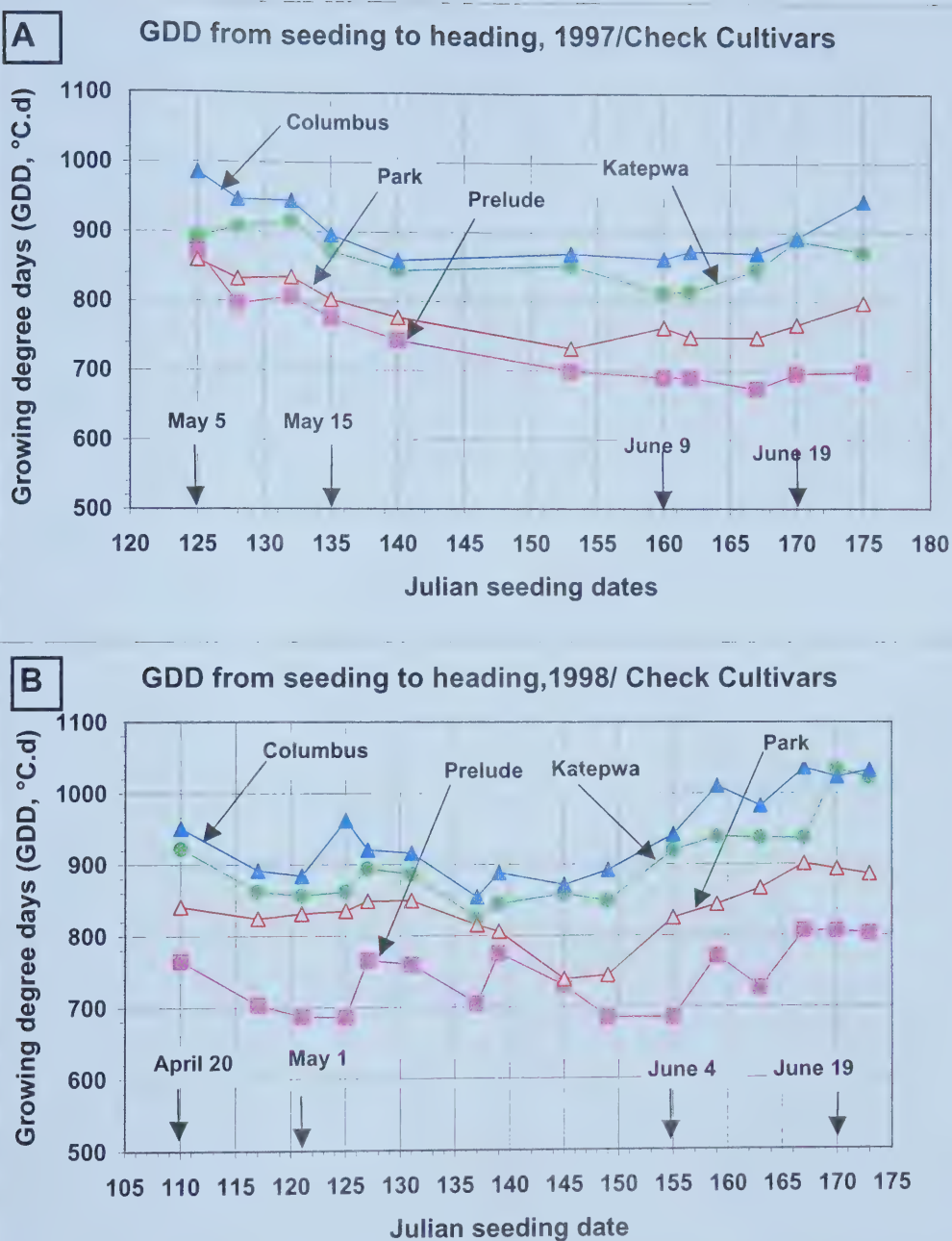


Fig. 4.1. The effect of seeding dates on growing degree-days (GDD) required for heading in check cultivars in 1997 (A) and in 1998 (B) at Edmonton Research Station.

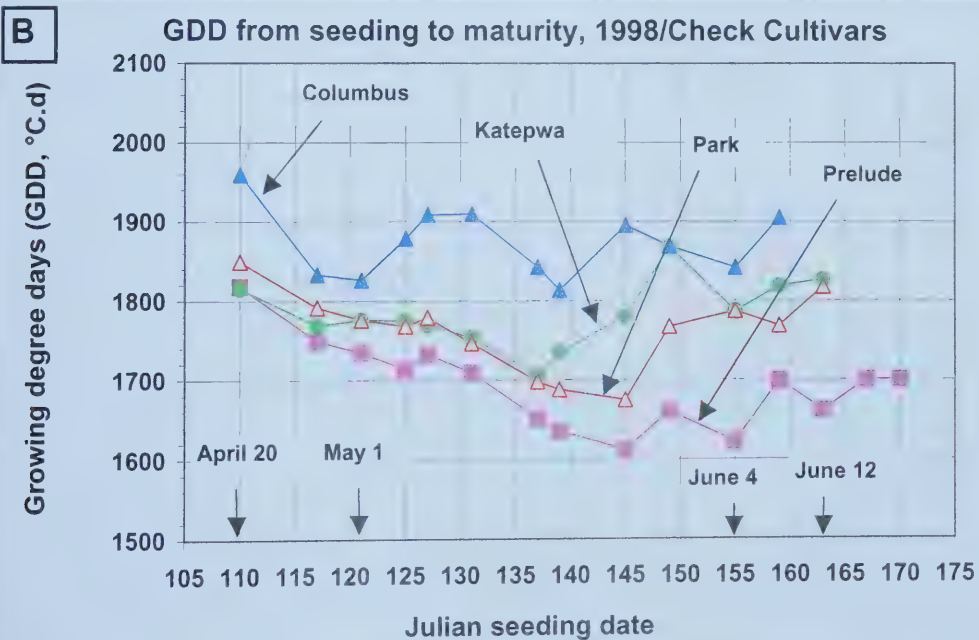
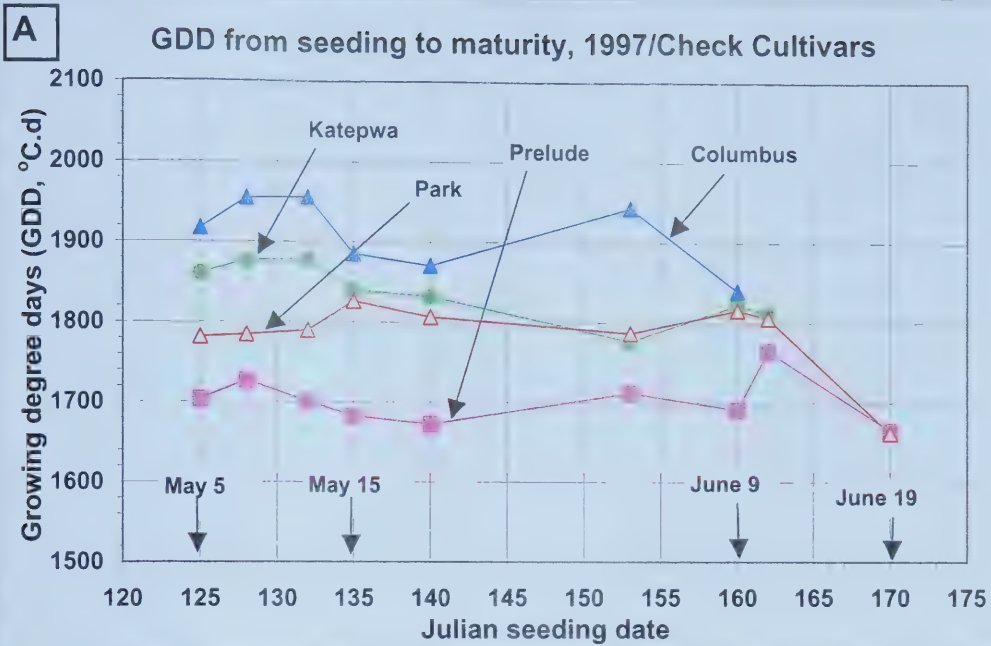


Fig. 4.2. The effect of seeding dates on growing degree-days (GDD) required for maturity in check cultivars in 1997 (A) and in 1998 (B) at Edmonton Research Station.

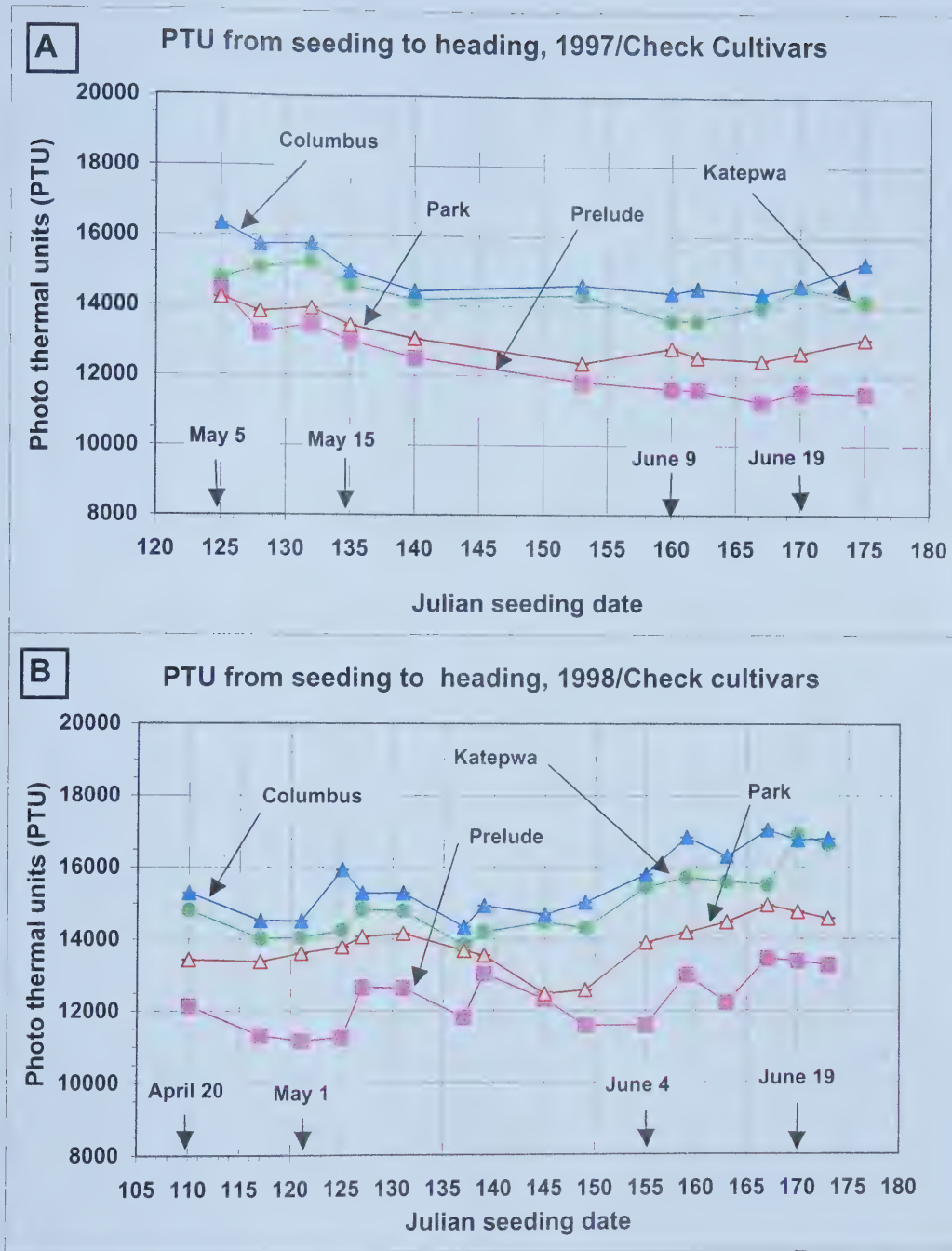


Fig. 4.3. The effect of seeding dates on photo-thermal units (PTU) required for heading in check cultivars in 1997 (A) and in 1998 (B) at Edmonton Research Station.

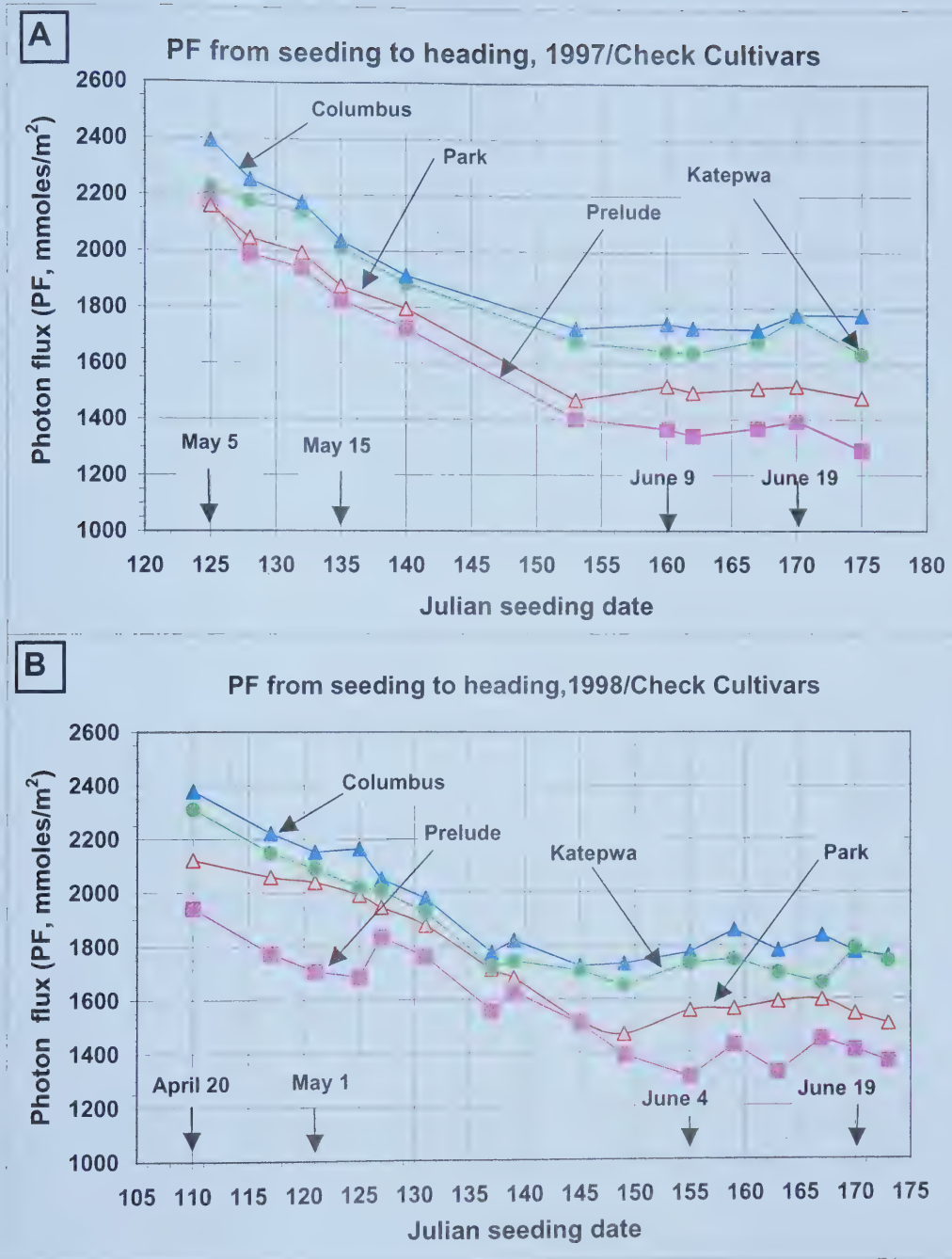


Fig. 4.4. The effect of seeding dates on photon flux (PF) required for heading in check cultivars in 1997 (A) and in 1998 (B) at Edmonton Research Station.

4.3.1.2. Group 5 chromosome substitution lines:

There was no apparent effect of GDD in heading for 1997 (Fig. 4.5A) in 5A substituted lines but increased GDD requirement for heading was observed in the later sowing dates of 1998 (Fig. 4.5B). CR5B required reduced GDD and headed earlier during the study period compared to Rescue, RC5B and Cadet in both years (Fig. 4.6 A, B), while during maturity no consistent pattern was observed. The substitution line 5D did not show significant sowing effect for heading (Fig. 4.7A) but a slightly higher requirement of GDD was observed in the later sowing dates of 1998 (Fig. 4.7B).

The PTU response of the chromosome substitution lines and their recipient parents were parallel to GDD requirement for heading and maturity for all years studied. Thus, further results on the PTU requirement is not reported here.

There was a consistent reduction in PF requirement in later sowing dates for heading (Fig. 4.8A, B) for all years. CR5A did not head in 1996, and headed only in the first two sowing dates in 1997 (Fig. 4.8A), but formed heads mostly in 1998 (Fig. 4.8B). It was observed that RC5A required less PF to head followed by Rescue, Cadet, and CR5A respectively (Fig. 4.8B). In the later June 22 sowing date of 1998, RC5A required 1600 mmol/m² PF to head followed by Rescue 1750 mmol/m² PF, Cadet 2000 mmol/m² PF and CR5A 2550 mmol/m² PF to head (Fig. 4.8B). Overall, the later seeding dates tended to require less PF for heading compared to earlier ones in 1997, 1998 (Fig. 4.9A, B) and for maturity.

CR5B required less PF for heading and maturity in all 3 years followed by Rescue, Cadet and RC5B respectively. Later sowing dates required a reduced amount of PF for 5D heading (Fig. 4.10A, B) in all years. RC5D and Rescue required similar and reduced PF in most of the sowing dates compared to Cadet and CR5D (Fig. 4.10A, B), while maturity showed exactly similar trends as observed for heading.

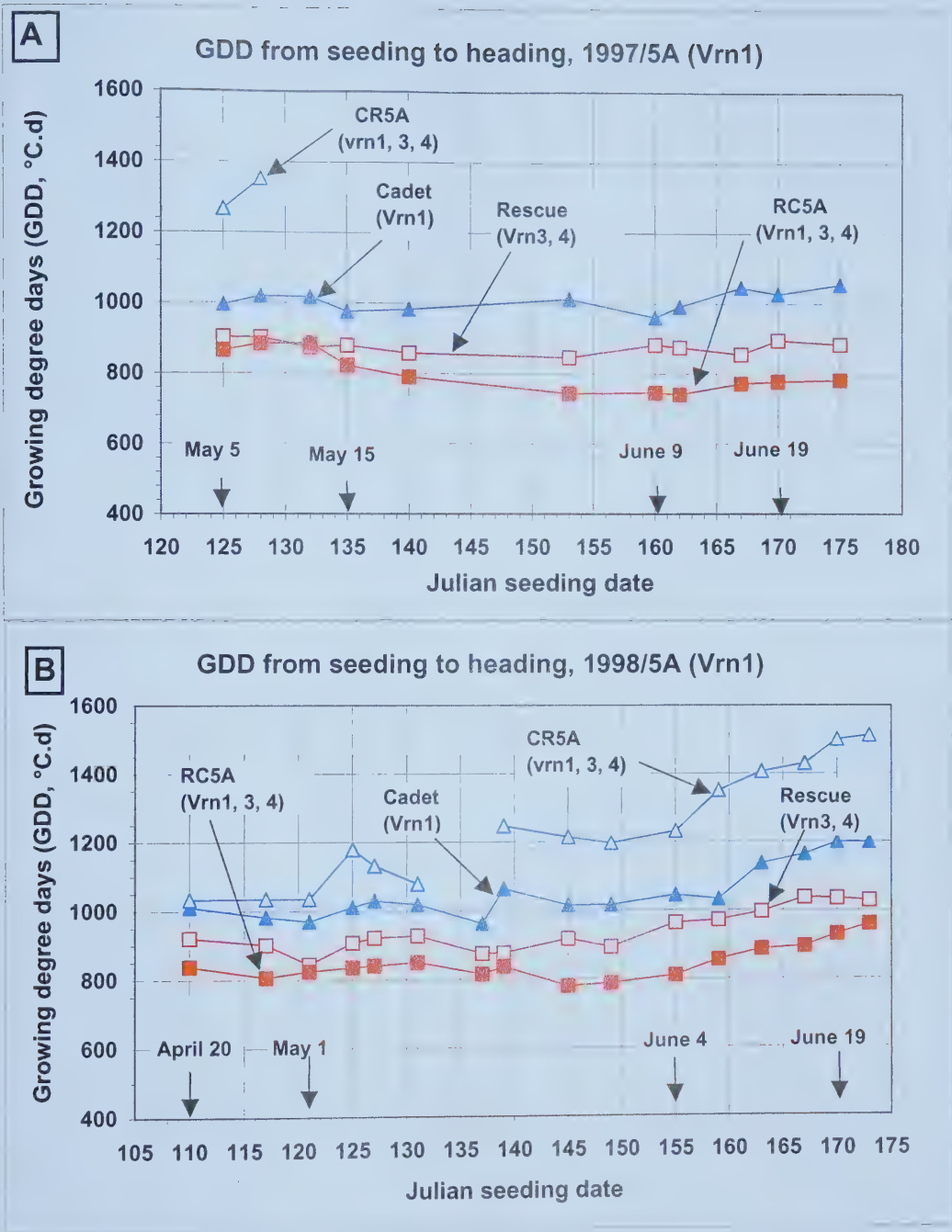


Fig. 4.5. The effect of seeding dates on growing degree-days (GDD) required for heading in 1997 (A) and in 1998 (B) in chromosome 5A substitution lines and their recipient parents at Edmonton Research Station.

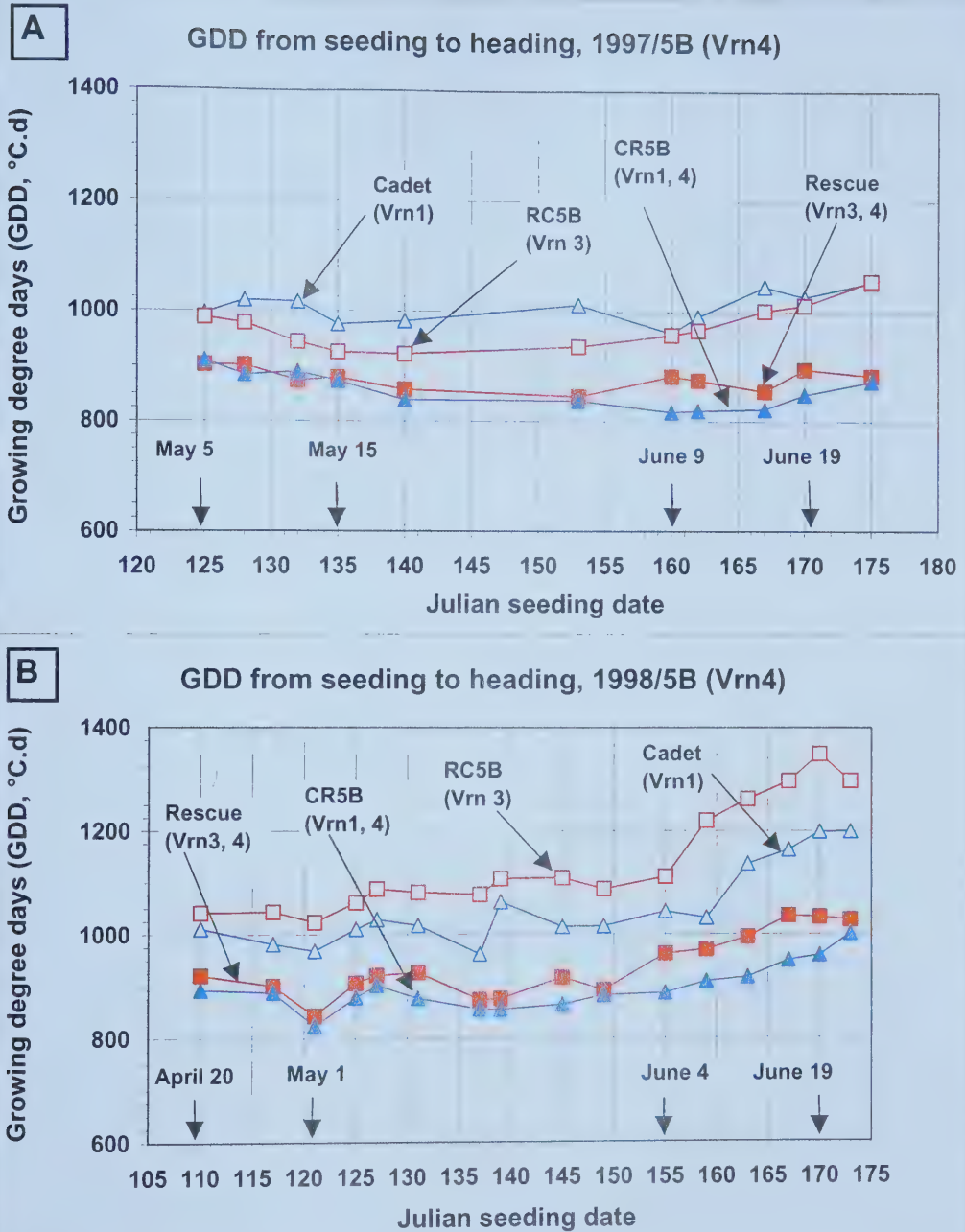


Fig. 4.6. The effect of seeding dates on growing degree-days (GDD) required for heading in 1997 (A) and in 1998 (B) in chromosome 5B substitution lines and their recipient parents at Edmonton Research Station.

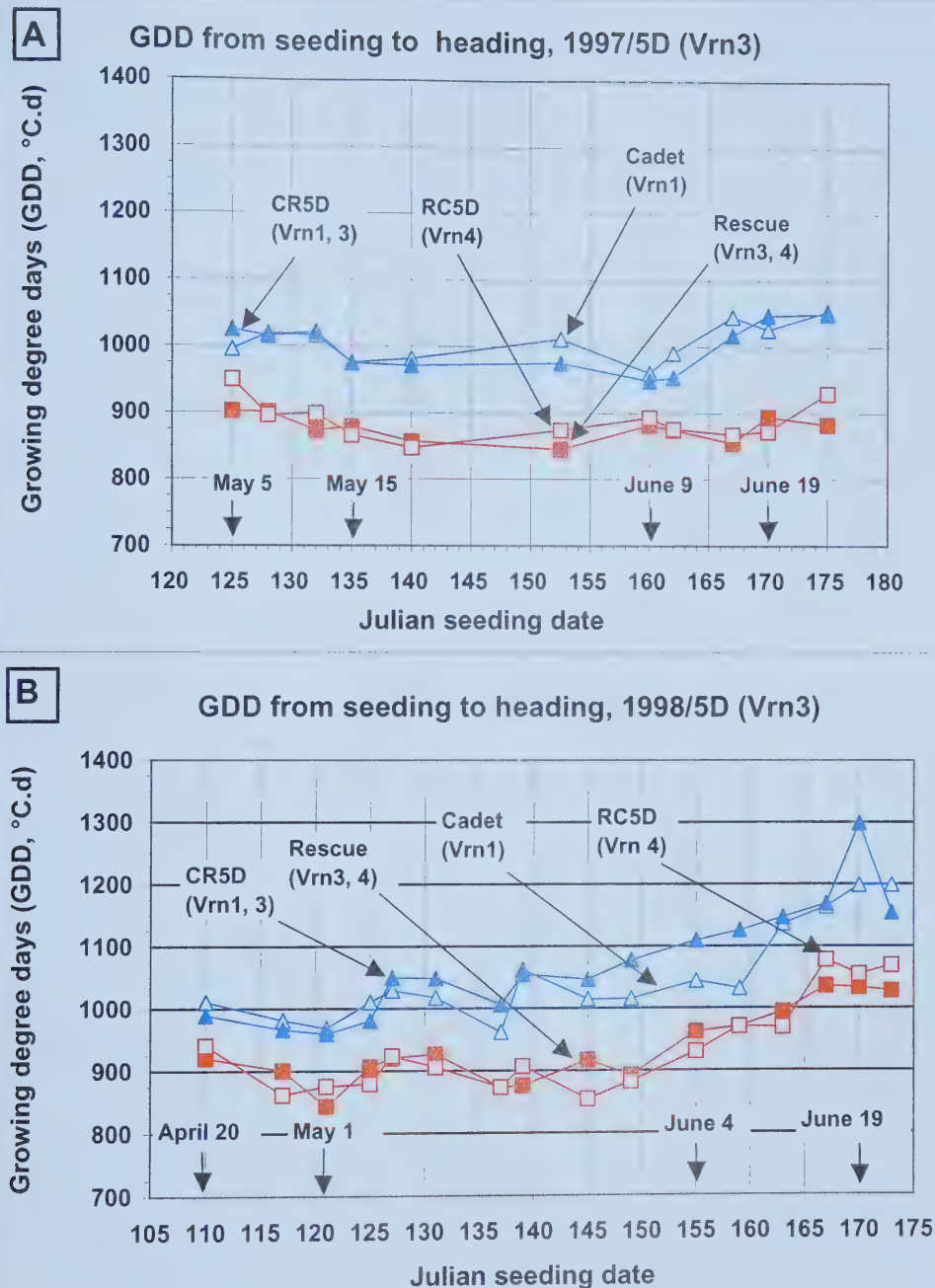


Fig. 4.7. The effect of seeding dates on growing degree-days (GDD) required for heading in 1997 (A) and in 1998 (B) in chromosome 5D substitution lines and their recipient parents at Edmonton Research Station

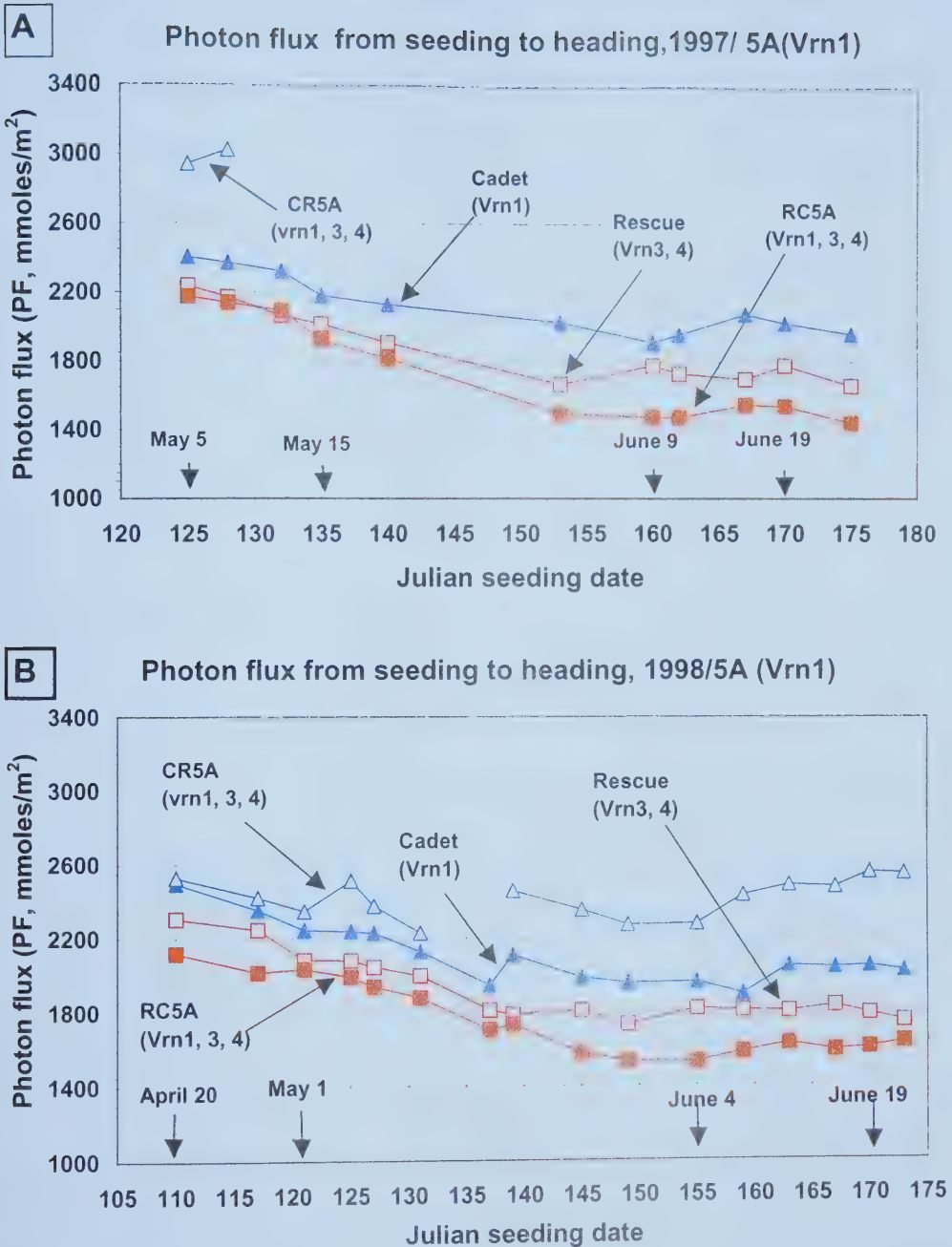


Fig. 4.8. The effect of seeding dates on photon-flux (PF) required for heading in 1997 (A) and in 1998 (B) in chromosome 5A substitution lines and their recipient parents at Edmonton Research Station.

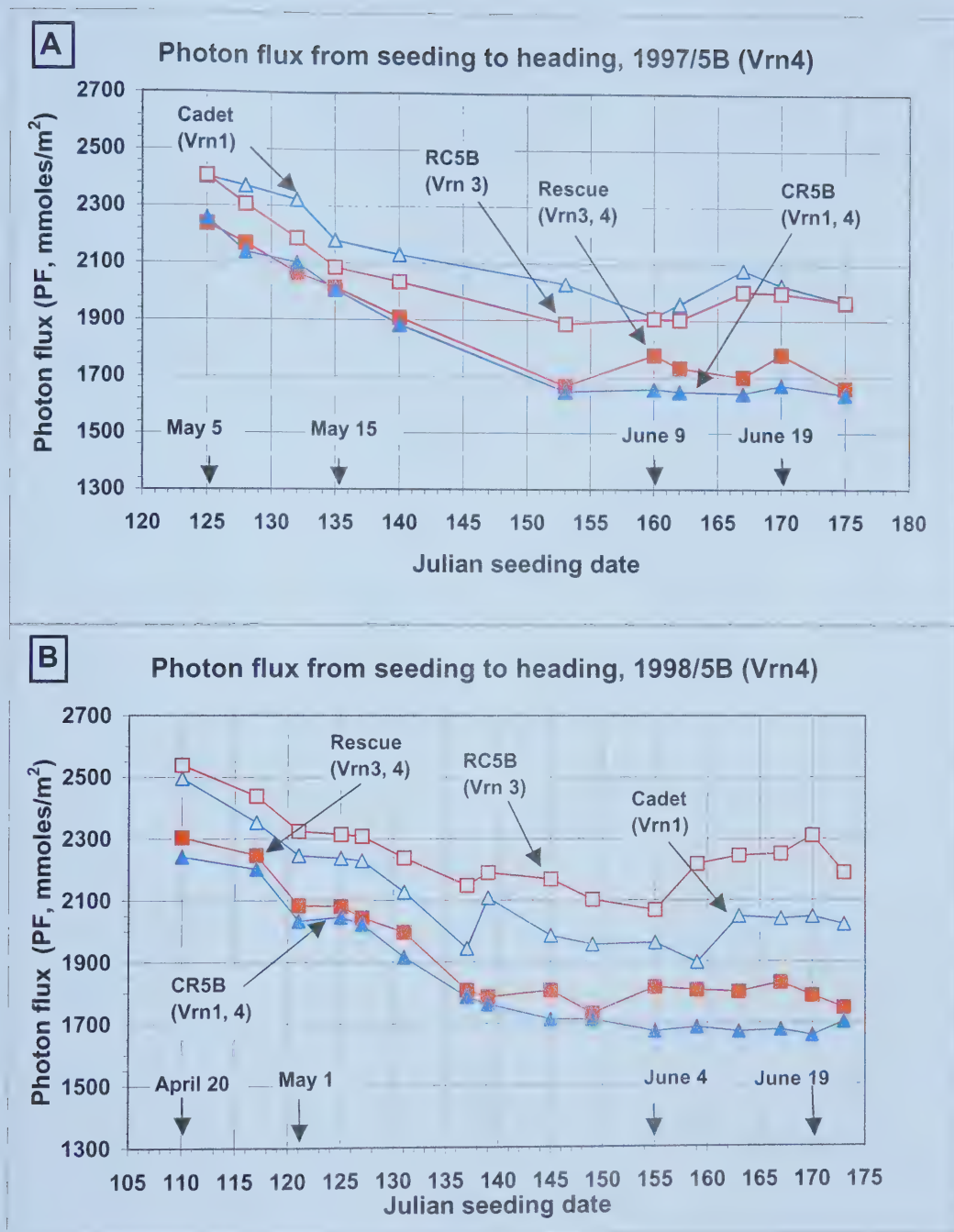


Fig. 4.9. The effect of seeding dates on photon-flux (PF) required for heading in 1997 (A) and in 1998 (B) in chromosome 5B substitution lines and their recipient parents at Edmonton Research Station.

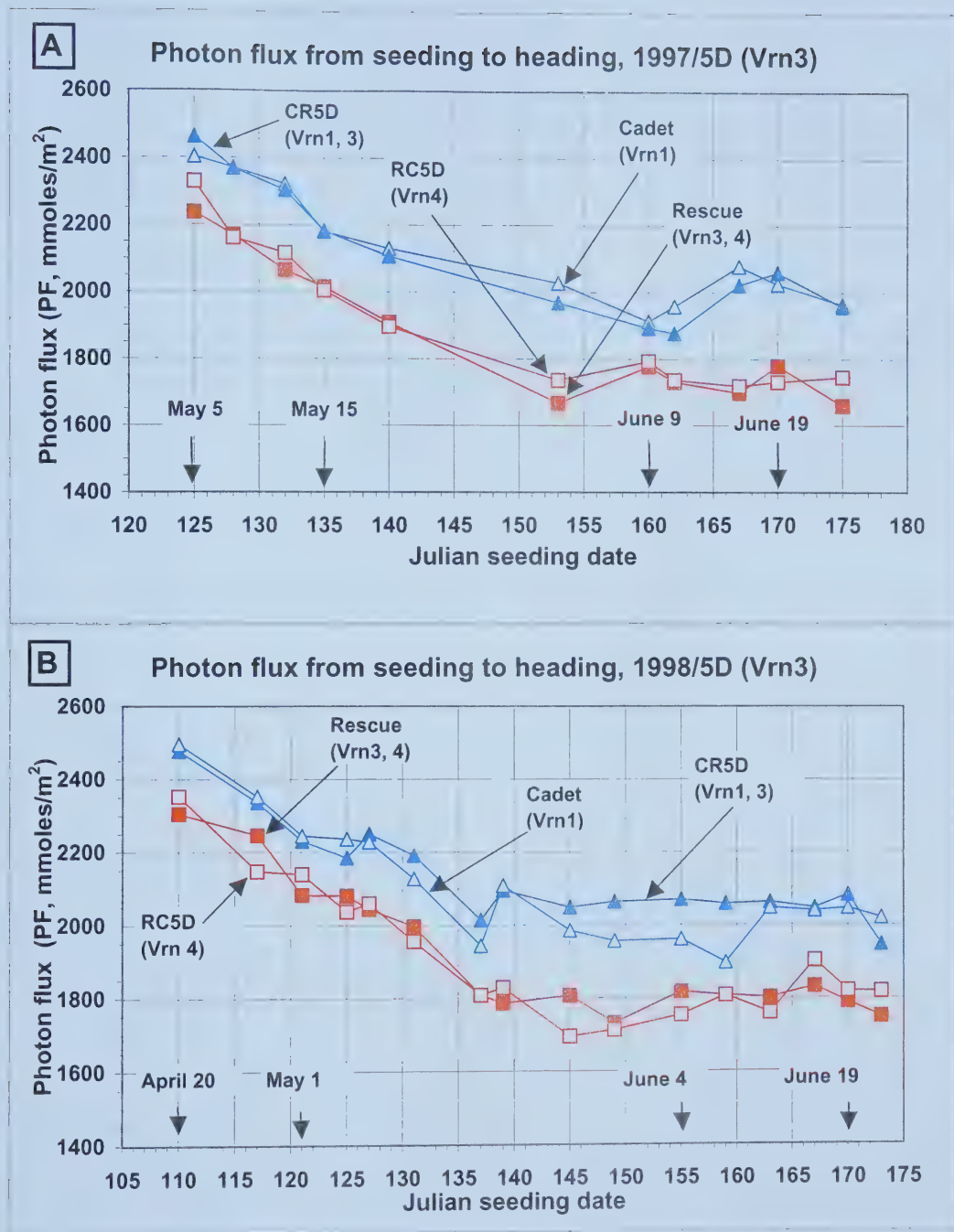


Fig. 4.10. The effect of seeding dates on photon-flux (PF) required for heading in 1997 (A) and in 1998 (B) in chromosome 5D substitution lines and their recipient parents at Edmonton Research Station.

4.3.2. Genetic effects of group 5-substituted chromosome

Effects of the substituted chromosomes shows differences in the amount of GDD requirement between substituted lines of 5A, 5B and 5D for heading and maturity in both Rescue and Cadet backgrounds (Table 4.1-4.7).

4.3.2.1. Growing degree-days (GDD)

In 1996, CR5B required less of GDD for heading (May 25; -218 °C.d) compared to its recipient parent Cadet (Table 4.1). In 1997, 1998 the introduction of both RC5B and CR5B resulted in a significant reduction in GDD requirement (Table 4.2, 4.3). In terms of maturity, the introduction of 5B resulted in a significant reduction of GDD requirement, but there was no consistent effect of the background in 1997 and 1998 (Table 4.4, 4.5). In the case of heading to maturity, the GDD requirement due to the introduction of the 5B chromosome showed no consistent trend in either 1997 or 1998 (Table 4.6, 4.7).

The presence of *Vrn1* carried by the Cadet 5A chromosome resulted in reduced GDD requirement for heading in all 3 years (Table 4.1- 4.3). The heading data for CR5A for 1996 and 1997 was unavailable because CR5A failed to head as expected. There was an apparent reduction in GDD requirement for maturity in both years (Table 4.4, 4.5), but in heading to maturity (Table 4.6, 4.7) no significant effect of GDD requirement was observed in 1998. However, in 1997 significant effects of reduced GDD requirement were observed in both backgrounds in a few cases.

The only significant difference on heading due to the presence of 5D was observed for the June 2, 1997, planting (Table 4.2). In the remainder of cases the chromosome introduction either increased or decreased GDD requirement in both Rescue or Cadet background, and these effects were not statistically significant (Table 4.1, 4.3). Similar inconsistency in the GDD requirement was observed for maturity (Table 4.4, 4.5) and heading to maturity (Table 4.6, 4.7).

Table 4.1. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days ($^{\circ}\text{C.d}$) required for heading in multiple planting dates at Edmonton Research Station in 1996.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date:						
May 13	- [†]	-81.3	-117.4*	-55.7	-26.4	-8.0
May 16	-	-40.3	-128.1	-49.5	14.6	18.0
May 19	-	-123.3*	-140.9*	-11.8	0.1	0.0
May 22	-	-2.8	-106.8	-113.2	67.4	11.4
May 25	-	-88.0	-218.0*	-30.9	-19.1	27.5
May 29	-	-102.9	-137.0*	-50.2	-0.9	21.8
May 31	-	-153.0*	-110.3*	-48.4	-9.4	18.5
June 3	-	-118.7	-33.4	-43.6	0.0	0.0
June 6	-	-90.5	-40.0	-40.6	6.9	-5.8
June 12	-	-64.9*	-117.0	-68.0	10.1	7.7

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences ($P < 0.05$).

Table 4.2. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days (°C.d) required for heading in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-271.1	-37.5	-85.5	-85.8	30.1	-47.8
May 8	-333.4	-17.9	-135.4*	-76.4*	-3.7	5.6
May 12	- [†]	-11.4	-127.1*	-70.0	5.1	-25.1
May 15	-	-57.6*	-103.2*	-46.4*	-1.1	12.7
May 20	-	-68.1*	-143.3*	-64.7*	-10.5	9.8
June 2	-	-101.7*	-174.1*	-90.6*	-36.2*	-29.2*
June 9	-	-136.0*	-143.4*	-75.3	-11.5	-11.5

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.3. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days (°C.d) required for heading in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-22.2	-83.2*	-117.8	-120.6	-21.4	-20.0
April 27	-52.9	-95.0*	-93.7	-143.2*	-15.2	38.9
May 1	-66.3	-19.4	-145.0*	-180.5*	-9.1	-32.4
May 5	-168.6	-71.7	-131.6	-155.2	-29.8	26.9
May 7	-101.8	-81.6	-127.0*	-167.2*	20.9	-2.9
May 11	-60.1	-77.4*	-140.9	-154.8*	30.7	20.6
May 17	-	-59.5	-106.4*	-203.0	44.5	-
May 19	-184.3*	-40.4	-207.7*	-231.5*	-7.5	-31.2
May 20	10.0	-48.7	7.1	-191.1	93.6	-0.1
May 25	7.9	-144.8*	-186.5*	-204.2*	-165.2	-59.0
May 29	-199.1	-138.6	-150.2	-192.2	31.7	62.5
June 4	-180.2*	-105.3	-133.5*	-195.4*	61.4	9.7
June 8	-186.8*	-153.5*	-157.4	-148.6	64.8	31.8

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

Table 4.4. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days ($^{\circ}\text{C.d}$) required for maturity in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-341.8	-127.2	-53.4	-157.8	130.3	83.4
May 8	-169.8*	-49.3*	-154.5*	-161.6*	17.5	-6.3
May 12	- [†]	-34.5	-89.2	-140.4	10.1	42.7
May 15	-	-106.6*	-109.0*	-115.5*	42.2*	-14.8
May 20	-	-57.6	-141.6*	-91.0*	27.1	-18.4
June 2	-	-88.6*	-235.6*	-16.8	-88.2	85.4*
June 9	-	-26.0	-	-	-	-4.5

[†] No comparison possible due to absence of heading, * indicates significant differences ($P < 0.05$).

Table 4.5. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days ($^{\circ}\text{C.d}$) required for maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-20.6	-42.1	-73.3	-73.3	-	32.1
April 27	-65.6	-11.5	-115.5*	-158.8*	-63.7*	21.6
May 1	-100.8	-20.6*	-97.6*	-156.4	-	-20.6
May 5	-184.6	-56.4	-70.0*	-120.0	77.6	-7.4
May 7	-238.0	-7.1	-100.8*	-194.2*	18.0	-96.4
May 11	-267.0	-2.9	-205.0*	-230.6*	10.4	32.8
May 17	-248.8	-102.8	-70.3	-149.3*	17.0	26.0
May 19	-206.4	-151.0	-113.1	27.5	-	114.5
May 20	78.7	6.1	-140.2	-156.2*	-99.1	-28.4
May 25	-	-52.8	-117.8*	-160.0	-50.8	-8.7
May 29	-47.1	-7.0	-169.5*	-184.5	24.3	-35.7
June 4	-	-54.2	-66.0	-109.5	17.4	16.1
June 8	-	-2.8	-94.0*	-	-	46.8

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences ($P < 0.05$).

Table 4.6. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days ($^{\circ}\text{C.d}$) required for heading to maturity in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-70.7	-64.5	32.1	-72.1	100.3	131.2
May 8	140.8*	-31.4	-19.0	-85.3*	21.2	-10.9
May 12	- [†]	-58.3	41.7	-58.6	5.0	86.8
May 15	-	-44.7	-5.8	-73.4*	43.3*	-32.0
May 20	-	10.5	1.6	-26.4	37.6	-28.1
June 2	-	10.6	-48.3	67.3	-40.7	114.5*
June 9	-	88.9*	-	-	-	22.5

[†] No comparison possible due to absence of heading, * indicates significant differences ($P < 0.05$).

Table 4.7. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on growing degree-days ($^{\circ}\text{C.d}$) required for heading to maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	1.6	41.1	44.5	47.4	21.4	52.1
April 27	-12.8	83.6	-21.8	-15.6	-48.5	-17.4
May 1	-34.5	-1.2	47.3	24.1	9.1	11.9
May 5	-16.0	15.3	61.7	35.2	107.3*	-34.3
May 7	-226.2	74.5	26.2	-27.0	-2.9	-93.5
May 11	-	80.3	-64.1	-75.8	-20.3	-53.4
May 17	-	-43.3	36.1	70.8	-27.5	26.0
May 19	-43.0	-110.6	94.6	259.0*	7.5	145.8*
May 20	68.7	54.8	-147.3	34.9	-192.8	-28.4
May 25	-	92.1	68.6*	27.4	144.4	50.2
May 29	-121.2	131.7	-19.3	7.7	-7.4	-98.2
June 4	-	51.1	67.6	85.9	-44.0	6.4
June 8	-	150.7	63.4	-	-	15.0

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences ($P < 0.05$).

4.3.2.2. Photo-thermal units (PTU)

The impact of *Vrn4* on PTU requirement for heading, maturity and heading to maturity, was similar to the GDD requirement for the three years (Table 4.8 - 4.14). There was an exception in the 1997 heading to maturity data, where CR5B showed an increased requirement of PTU (Table 4.13). In 1998 the maturity of CR5A did not require an increase in the amount of PTU (Table 4.12) and in heading to maturity in 1998 (Table 4.14) RC5A showed an increased amount of PTU requirement. The PTU requirements for 5D follow the same pattern as GDD for heading and maturity, with the exception of 1997 heading to maturity, where it needed increased amount of PTU in RC5D (Table 4.13).

4.3.2.3. Photon flux (PF)

The PF requirement for heading was significantly reduced in CR5B for 1996, and for both CR5B and RC5B for 1997 and 1998 (Table 4.15-4.17). In 1997, 1998 maturity, both backgrounds Rescue and Cadet of 5B required reduced amounts of PF to mature (Table 4.18, 4.19). Only, RC5B showed significantly less PF requirement for heading to maturity in a few sowing dates (Table 4.20, 4.21).

Generally there was a reduction in PF requirement for heading and for maturity (Table 4.18, 4.19) in both backgrounds, in all studied years. The only significant difference on heading due to the introduction of 5D was observed in the June 2 1997, planting (Table 4.16). In rest of the cases the chromosome introduction either increased or decreased PF requirement in both Rescue or Cadet background and these effects were statistically not significant (Table 4.15 – 4.17). Similar inconsistency in the PF requirement was also observed for maturity (Table 4.18 – 4.20) and heading to maturity (Table 4.21).

4.3.3. Growth Chamber studies

Phenotyping check cultivars and substituted lines under growth chamber conditions gave parallel results as in the field. Prelude formed heads earliest (with a

Table 4.8. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for heading in multiple planting dates at Edmonton Research Station in 1996.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date:						
May 13	- [†]	-1349.0	-1928.7*	-914.9	-433.2	-132.3
May 16	-	-673.3	-2106.9	-909.1	236.5	297.1
May 19	-	-2039.6*	-2302.1*	-191.1	2.1	0.0
May 22	-	-53.9	-1722.3	-1830.4	1075.7	184.7
May 25	-	-1421.0	-3501.5*	-493.8	-302.3	442.5
May 29	-	-1655.4	-2192.3*	-800.7	-14.7	350.0
May 31	-	-2455.8*	-1759.6*	-754.5	-148.3	292.0
June 3	-	-1892.8	-521.8	-669.2	0.0	0.0
June 6	-	-1425.3	-609.4	-615.1	104.8	-87.3
June 12	-	-988.4*	1788.1	-1043.2	153.4	117.3

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences ($P < 0.05$).

Table 4.9. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for heading in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-4423.5	-628.7	-1424.8	-1426.5	498.0	-799.1
May 8	-5371.4	-298.8	-2250.8*	-1271.4*	-62.6	75.5
May 12	- [†]	190.7	-2103.3*	-1163.7	82.3	-418.4
May 15	-	-957.9*	-1703.8*	-769.7*	-17.4	211.3
May 20	-	-1131.4*	-2358.1*	-1065.1*	-170.7	162.0
June 2	-	-1665.7*	-2782.9*	-1450.8*	-572.3*	-470.1*
June 9	-	-2173.2*	-2274.3*	-1186.8*	-181.8	-182.3

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.10. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for heading in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-377.7	-1406.9*	-1984.9	-2037.7	-364.2	-336.3
April 27	-892.7	-1604.4*	-1586.6	-2423.5*	-257.8	657.0
May 1	-1113.9	-328.5	-2454.9*	-3053.2*	-153.7	-551.0
May 5	-2797.9	-1212.4	-2215.3	-2604.5	-498.7	453.6
May 7	-1692.5	-1376.9	-2132.4*	-2797.7*	348.0	-47.5
May 11	-996.0	-1302.9*	-2361.0	-2580.6*	507.6	346.4
May 17	-	-1000.0	-1778.1*	-3366.5	735.0	-
May 19	-2978.8*	-675.6	-3438.0*	-3814.6*	-122.4	-522.4
May 20	167.8	-828.0	122.9	-3219.8	1581.0	-2.2
May 25	135.3	-2455.2*	-3134.6*	-3414.5*	-2779.1	-991.0
May 29	-3194.3	-2293.2	-2459.5	-3126.5	510.6	1027.4
June 4	-2864.8*	-1731.1	-2168.4*	3157.0*	980.6	158.0
June 8	-2919.3*	-2488.2*	-2524.0	-2355.6	1022.6	511.6

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

Table 4.11. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for maturity in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-4091.2	-1761.7	-661.6	-2103.0	167.5	1128.7
May 8	-1881.9*	-681.4*	-2017.3*	-2133.6*	219.8	-87.6
May 12	- [†]	-456.7	-1109.3	-1777.4	112.8	563.2
May 15	-	-1438.3*	-1349.5*	-1468.0*	431.5*	-200.2
May 20	-	-731.0	-1631.9*	-1096.1*	293.0	-269.7
June 2	-	-974.7*	-2544.4*	-168.9	-901.6	951.5*
June 9	-	-248.5	-	-	-	-45.9

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.12. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-312.5	-647.0	-1121.4	-1117.9	-	493.1
April 27	-952.7	-177.3	-1740.6*	-2377.5*	-949.8*	332.7
May 1	-1469.5*	-316.0	-1451.5*	-2317.4	-	-312.4
May 5	-2643.6	-845.3	-1019.1*	-1737.1	1135.8	-107.6
May 7	-3257.0	-101.1	-1468.0*	-2785.1*	257.6	-1397.0
May 11	-3570.1	33.8	-2966.6*	-3269.0*	148.4	474.6
May 17	-3307.0	-1483.0	-989.9	-2065.7*	233.3	366.4
May 19	-2591.3	-2108.5	-1554.9	377.9	-	1587.1
May 20	1110.5	97.5	-1999.0*	-2242.2*	-1410.9	-416.1
May 25	-	-764.7	-1680.4*	-2251.1	-710.5	-123.8
May 29	-563.9	-81.3	-2216.3	-2409.8	276.8	-494.2
June 4	-	-697.1	-787.0*	-1242.7	150.5	210.7
June 8	-	-47.5	-965.2	-	-	561.1

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

Table 4.13. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for heading to maturity in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	243.1	-798.8	773.2	-633.7	1172.4	1920.4
May 8	2933.9*	-372.5	255.3	-835.1	281.4	-164.9
May 12	- [†]	-854.7	1044.8*	-408.7	46.1	1304.3
May 15	-	-384.3	322.6	-777.5	473.4	-478.3
May 20	-	-388.8	635.7*	-65.1	474.1	-422.0
June 2	-	-564.4	239.9	1179.4	-261.0	1467.7*
June 9	-	-1519.5*	-	-	-	362.0

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.14. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photo-thermal units required for heading to maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	74.1	783.4	901.4	959.2	370.2	830.5
April 27	-37.3	1445.1	-116.2	101.4	-676.9	-333.3
May 1	-329.0	18.2	1046.9*	795.7	155.1	247.8
May 5	199.8	388.3	1220.4	898.2	1619.9*	-564.1
May 7	-2684.0	1287.8	688.4	48.6	-92.4	1336.0
May 11	-	1341.7*	-576.5	-639.7	-371.8	-816.5
May 17	-	-468.4	796.0	1556.1	-521.3	363.5
May 19	-12.3	-1419.0	1910.8	4212.4*	125.5	2095.1*
May 20	933.9	932.3	-2108.6	1001.6	-2984.6	-410.2
May 25	-	1718.4	1465.8*	912.3	2072.6	868.1
May 29	2075.0	2230.8	241.7	678.0*	-210.6	-1522.4
June 4	-	1026.6	1327.7	1804.8	-781.2	53.4
June 8	-	2405.7	1410.9	-	-	76.1

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

Table 4.15. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for heading in multiple planting dates at Edmonton Research Station in 1996.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date:						
May 13	- [†]	-111.4	-238.4*	-125.5	-64.4	-22.3
May 16	-	-47.1	-260.1	-109.7	32.3	40.9
May 19	-	-219.5*	-317.8*	-25.2	0.1	0.0
May 22	-	-3.0	-232.3	-260.7	131.2	24.8
May 25	-	-203.3	-440.0*	-44.2	-29.1	63.5
May 29	-	-223.9	-268.9*	-84.5	-13.5	36.4
May 31	-	-308.5*	-190.4*	-109.0	-18.4	26.2
June 3	-	-210.1	-84.7	-120.8	0.0	0.0
June 6	-	-815.4	-118.9*	-116.0	16.9	-20.7
June 12	-	-178.1*	-230.4	-115.3	-15.6	20.9

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences ($P < 0.05$).

Table 4.16. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for heading in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-538.8	-62.7	-145.8	-169.3*	59.7	-91.8
May 8	-654.0	-30.5	-233.1*	-137.7*	-2.2	7.8
May 12	- [†]	29.9	-221.7*	-122.3	-18.5	-51.7
May 15	-	-91.3*	-174.1*	-70.7*	2.9	9.2
May 20	-	-92.7*	-246.4*	-126.8*	-22.8	11.4
June 2	-	-167.0*	-380.0*	-219.8*	-58.8*	-69.1*
June 9	-	-302.0*	-255.8*	-129.5*	-20.2	-16.5

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.17. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for heading in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-30.7	-186.2	-254.6*	-234.6	-18.6	-48.6
April 27	-67.2	-229.7*	-151.7	-191.8	-16.6	98.7
May 1	-102.7	-48.5	-212.4*	-241.2*	-13.8	-56.6
May 5	-274.6	-91.1	-191.7	-232.9	-52.1	43.3
May 7	-145.6	-107.6	-206.0*	-263.8*	22.3	-15.9
May 11	-101.0	-119.8	-211.7	-240.8*	64.5	41.3
May 17	-	-104.3	-157.3*	-340.1	70.4	-
May 19	-347.3*	-54.0	-344.7*	-404.0*	-12.8	-43.2
May 20	18.5	-64.9	3.7	-280.7	134.3	4.1
May 25	2.3	-176.9*	-276.0*	-321.7*	-231.4	-94.1
May 29	-367.5	-230.7	-271.3	-362.2	63.1	111.2
June 4	-314.4*	-197.0*	-243.4*	-370.5*	107.8	18.7
June 8	-315.3*	-285.4*	-287.3	-251.3	108.3*	63.3

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

Table 4.18. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-552.2	-240.6	-96.1	-276.2	257.9	160.0
May 8	-260.0*	-92.7*	-283.5*	-301.2*	30.4	-14.1
May 12	- [†]	-72.0	-178.5	-260.7	21.3	85.1
May 15	-	-208.2*	-215.6*	-212.1*	86.5*	-24.7
May 20	-	-111.0	-247.2*	-177.9*	44.2	-31.2
June 2	-	-131.4*	-361.8*	-8.7	-145.2	152.1*
June 9	-	-43.6	-	-	-	-5.7

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.19. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-36.3	-71.2	-115.3	-122.8	-	50.2
April 27	-107.3	-21.5	-181.3*	-248.0*	-98.4	37.1
May 1	-56.6*	-28.8	-157.6*	-250.7	-	-36.3
May 5	-302.6	-91.9	-106.4*	-192.4	119.0	-4.9
May 7	-412.4	-11.2	-156.6*	-324.3*	34.2	-158.1
May 11	-445.8	6.6	-340.4*	-388.3*	16.9	-53.6
May 17	-415.1	-169.0	-127.1	-251.0	33.0	49.2
May 19	-350.6	-252.7	-196.8	48.3	-	194.6
May 20	141.8	7.8	-240.0*	-260.8*	-171.1	-45.2
May 25	-	79.8	-202.6*	-284.8	-92.9*	-18.3
May 29	-79.1	-9.1	-278.6	-299.0	46.8	-63.0
June 4	-	-89.5	-115.8*	-184.2	26.0	24.3
June 8		-1.0	-160.0	-	-	78.1

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

Table 4.20. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for heading to maturity in multiple planting dates at Edmonton Research Station in 1997.

Chromosome substituted:	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
May 5	-10.0	-125.7	49.5	-106.1	197.5	251.1
May 8	-345.6*	-61.7	-50.3	-163.1*	32.4	-21.9
May 12	- [†]	-127.2	49.3	-113.7	40.0	177.3
May 15	-	-109.6*	-40.9	-148.5*	83.1*	-41.2
May 20	-	-18.6	0.5	-50.2	66.9	-42.6
June 2	-	30.2	42.7	223.4*	-67.4	220.8*
June 9	-	224.5*	-	-	-	35.4

[†] No comparison possible due to absence of heading, * indicates significant differences (P<0.05).

Table 4.21. Differences between recipient parents and substituted chromosomes 5A, 5B or 5D in Cadet or Rescue background on photon flux required for heading to maturity in multiple planting dates at Edmonton Research Station in 1998.

Chromosome substituted :	<u>5A (Vrn1)</u>		<u>5B (Vrn4)</u>		<u>5D (Vrn3)</u>	
Background :	Cadet	Rescue	Cadet	Rescue	Cadet	Rescue
Seeding date :						
April 20	-5.5	115.0	139.3	111.8	18.6	98.8
April 27	-40.1	208.2	-29.6	-56.2	-81.9	-61.5
May 1	-53.9	19.8	54.8	-9.5	13.8	20.3
May 5	-27.5	-0.8	85.3	40.4	171.2*	-48.2
May 7	-413.0	96.4	49.3	-60.2	11.9	-142.2
May 11	-	126.4	-128.6	-147.3	-47.5	-95.0
May 17	-	-64.7	-30.2	120.8	-37.0	49.2
May 19	-43.1	-198.2	147.6	451.7*	12.7	237.2
May 20	123.2	72.7	-243.6	19.9	-305.4	-49.3
May 25	-	97.0	73.5	8.5	138.5	75.7
May 29	242.8	221.3*	-6.4	65.0	-16.7	-174.2
June 4	-	108.1	128.8	188.6	-82.4	5.4
June 8	-	285.5	129.5	-	-	14.2

[†] No comparison possible due to absence of heading in CR5A, * indicates significant differences (P<0.05).

658.8 \pm 13.8 °C.d, heat units) in terms of GDD requirement, while Columbus was the cultivar requiring the most heat units (1228.8 \pm 27.6 °C.d). Similar finding was observed in terms of PTU requirement with Prelude being the earlier one to mature, but the amount of PTU requirements are different from the field (Table. 4.22). The PF was calculated for the controlled environment but the results was the same as PTU (data not shown).

4.3.4. Interaction between seeding date and cultivars

The analysis of variance showed that there was significant interaction between the substitution lines and seeding dates in terms of GDD requirements in different years for heading and maturity. The data for all 3 years of heading and 2 years for maturity is shown in Table 4.23. Analysis of data in terms of seeding date, substitution lines, and seeding date x substitution lines interaction, showed the effect of the seeding date to be different for RC5A and Rescue in controlling heading in terms of GDD requirement. The most significant effect of seeding date was observed in the 5B substitution line in both backgrounds in the year 1997 heading and maturity and, in the 1998 heading data (Table 4.23). In the 5D substitution line of both backgrounds the significant effect of the seeding date was observed in 1997 and 1998 (Table 4.23) for heading.

Similar analysis on PTU revealed significant interaction between seeding date with respect to PTU requirements of the substitution lines for heading and for maturity (Table 4.24) also which is similar to GDD requirement. Thus, further data on PTU was not reported.

In the case of PF, analysis of variance shows significant interaction between seeding date and the 5B chromosome substitution lines in 1997 and 1998 for heading and for maturity 1997 (Table 4.25). The substitution lines of 5A and 5D do show some effect in the different seeding date for heading or maturity during the study period (Table 4.25). The results of the correlation matrix showed a very strong positive correlation (.99) between GDD and PTU. For PTU and PF or GDD and PF there was a moderately positive correlation (.71) (see examples in appendix, Table 7.7-7.8).

Table 4.22. Growing degree-days (GDD), and photothermal units (PTU) required for heading and maturity in spring wheat genotypes grown in growth chamber at 15°C/ 16 hr days, 10°C nights.

Cultivar	Heading		Maturity	
	GDD (\pm S.E.)	PTU (\pm S. E.)	GDD (\pm S.E.)	PTU (\pm S. E.)
Prelude	658.8 (13.8)	10540 (221)	1737.2 (60.4)	27800 (966)
Park	908.8 (27.7)	14540 (444)	2228.8 (41.9)	35660 (671)
Rescue	917.5 (18.0)	14680 (288)	2107.5 (52.1)	33720 (833)
RC5D	948.8 (19.3)	15180 (309)	2047.5 (64.3)	32760 (1029)
Katepwa	1111.3 (22.4)	17780 (359)	2292.5 (65.5)	36680 (1047)
CR5D	1203.8 (27.9)	19260 (446)	2300.0 (34.4)	36800 (550)
Cadet	1223.8 (22.7)	19580 (364)	2267.5 (20.1)	36280 (322)
Columbus	1228.8 (27.6)	19660 (441)	2358.8 (20.1)	37740 (321)

Table 4.23. Analysis of variance for growing degree-days (GDD) for 1996, 1997 and 1998 showing the effect of seeding date on chromosome substitution lines. (* indicate significant, and ns indicate non-significant difference at 5% level, – indicates missing data).

			1996	1997	1998
Cadet/ CR5A	DH	Seeding date	*	*	*
		Cultivar	ns	*	*
		S.date x Cultv.	ns	ns	*
	DM	Seeding date	-	ns	*
		Cultivar	-	ns	*
		S.date x Cultv.	-	ns	*
Rescue/ RC5A	DH	Seeding date	*	*	*
		Cultivar	*	*	ns
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns
Cadet/ CR5B	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	*	ns
Rescue/ RC5B	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	ns
		S.date x Cultv.	-	*	ns
Cadet/ CR5D	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	ns
		S.date x Cultv.	-	*	ns
Rescue/ RC5D	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	*	*	ns
	DM	Seeding date	-	*	*
		Cultivar	-	ns	*
		S.date x Cultv.	-	ns	*

Table 4.24. Analysis of variance for photo-thermal units (PTU) for 1996, 1997 and 1998 showing the effect of seeding date on chromosome substitution lines. (*indicate significant, and ns indicate non-significant difference at 5% level, – indicates missing data).

			1996	1997	1998
Cadet/ CR5A	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	ns	*
	DM	Seeding date	-	*	*
		Cultivar	-	ns	*
		S.date x Cultv.	-	ns	*
Rescue/ RC5A	DH	Seeding date	*	*	*
		Cultivar	*	*	ns
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns
Cadet/ CR5B	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	*	ns
Rescue/ RC5B	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	ns
		S.date x Cultv.	-	*	ns
Cadet/ CR5D	DH	Seeding date	*	*	*
		Cultivar	ns	ns	ns
		S.date x Cultv.	ns	ns	ns
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns
Rescue/ RC5D	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	ns
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns

Table 4.25. Analysis of variance for photon flux (PF) for 1996, 1997 and 1998 showing the effect of seeding date on chromosome substitution lines. (* indicate significant, and ns indicate non-significant difference at 5% level, – indicates missing data)

			1996	1997	1998
			Sig.	Sig.	Sig.
Cadet/ CR5A	DH	Seeding date	*	*	*
		Cultivar	ns	*	*
		S.date x Cultv.	ns	ns	*
	DM	Seeding date	-	*	*
		Cultivar	-	ns	*
		S.date x Cultv.	-	ns	*
Rescue/ RC5A	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	*	ns
Cadet/ CR5B	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	*	*	*
	DM	Seeding date	-	*	*
		Cultivar	-	*	*
		S.date x Cultv.	-	*	ns
Rescue/ RC5B	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	*
	DM	Seeding date	-	*	ns
		Cultivar	-	*	ns
		S.date x Cultv.	-	*	ns
Cadet/ CR5D	DH	Seeding date	*	*	*
		Cultivar	ns	ns	ns
		S.date x Cultv.	ns	ns	ns
	DM	Seeding date	-	*	*
		Cultivar	-	*	ns
		S.date x Cultv.	-	ns	ns
Rescue/ RC5D	DH	Seeding date	*	*	*
		Cultivar	ns	*	ns
		S.date x Cultv.	ns	*	ns
	DM	Seeding date	-	*	*
		Cultivar	-	ns	ns
		S.date x Cultv.	-	ns	ns

4.4. Discussion

The check cultivars exhibited a wide range of GDD, PTU and PF requirement for heading and maturity through all years studied. Late seeding dates induced early heading in check cultivars with a concomitant reduction of GDD. GDD is more normally used as the dependent variable in physiological studies (Wallace and Yan, 1998). The *Vrn* gene composition of the check cultivars is not known, but the ranking of the cultivars for heading and maturity parallels that reported in the literature (DePauw et al. 1995). Results observed with PTU for heading and maturity was similar to that with GDD, which could be due to multiplying a fixed variable of temperature required for each cultivar with another fixed variable, the photoperiod. The analysis of data in terms of GDD allows one to evaluate the results in terms of the effects of temperature and duration, while PTU adds another variable (photoperiod) to the analysis. Since the photoperiod apparently did not have a significant effect in the present study, analysis based on GDD appeared to be more appropriate. The field study reveals the fact that heat unit accumulation of GDD, PTU and accumulated PF for heading and for maturity traits of check cultivars is in the same range as that of the substitution lines. Thus, field characterization of check cultivars suggests the possibility that a similar *Vrn* gene system might exist in these Canadian cultivars as in the substitution lines.

The Cadet/Rescue chromosome substituted lines was a suitable genetic system to study phenological development for heading and maturity that could explain the full range of differences in relation to temperature, photoperiod and solar radiation required. The present study showed that 5B substituted lines resulted in early heading and maturity, which could have been due to the presence of *Vrn4*, resulting in reduced heat unit (GDD) requirement, similar to that reported by Gardner et al. (1993).

Slafer and Rawson (1995) examined responses of genetic variation in growth and development of wheat due to temperature. Temperature and photoperiod have also been shown to interact in determining the phenological development of wheat (Wall and Cartwright 1974; Halloran and Pennell 1982). It is difficult in field studies to separate the influences of different environmental variables on growth rate and development because of the strong correlation between the length of the photoperiod and daily levels

of solar radiation (Flood and Halloran 1984). Since observations were not made on phenological developments prior to heading, it is not possible to determine if growth stages differed significantly between genotypes, or if each stage was slightly prolonged in later heading substituted lines, as has been reported earlier (Flood and Halloran 1984). If the photoperiod is the main factor controlling the ranking of cultivars within a temperature treatment, then the addition of temperature effects may alter the ranking of the phenotypes, but will do so within the limits established by photoperiod (Pirastén and Welsh 1980). In the present study, since photoperiod did not appear to be the determining factor, the temperature effect within the boundary established by photoperiod appears to be playing the major role in observed differences. According to Flood and Halloran (1984) if there are genetic differences in response to growth temperature in wheat, then the responses appeared to be strongest between 9 and 14°C, and in the present study the average temperature from April to July in 1998 was 14°C. As the later seeding dates resulted in earlier heading, this strongly supports the view that there is a differential effect of temperature on both heading and maturity (Flood and Halloran 1984).

According to Stelmakh (1998), 20-25% of the variation in heading date of wheat may be attributed to genetic differences in photoperiod gene system (photoperiodic response), however in the present case, PTU results show that photoperiod did not have any significant effect over different seeding dates. This result is similar to the finding of del Pozo et al. (1987), where absence of photoperiodic response to reach a particular developmental stage in spring wheat cultivar has been reported, and the temperature was observed to be the main controlling factor for development. Most likely in the present case, substitution lines, photoperiodic sensitivity, and vernalization requirement, contributed equally to the variation for heading and maturity during the study period, which agrees with the findings of Shindo and Sasakuma (1998). Another possible explanation could be the existing photoperiod in this northern latitude is well above the critical requirement, so that the variation in photoperiod above this threshold did not have any effect on the phenological development of wheat (Shaykewich 1995, Whisler et al. 1986). These results suggest that each chromosome pair substitution (5A, 5B and 5D) possibly has its unique controls and requirements in phenological development.

Although the PTU did not have any effect on heading and maturity, its requirement varied from sowing to heading and maturity at different seeding dates. The current method of calculating PTU ignores environmental interactions, although it is known that different growth phases in wheat vary in their responses to photoperiod (Kirby et al. 1985).

In the present study, replacing days elapsed from seeding to heading with PF, provided a clear picture of genotypic response of spring wheat development. In all three years, late seeding dates resulted in reduced PF requirement contributing to early heading in the substitution lines (5A, 5B and 5D). This is similar to the findings in terms of days elapsed for seeding to heading as reported in the previous chapter. Radiant energy in the form of photon flux may influence heading in the substitution lines, as has been shown for genotypes with mild responsiveness to photoperiod (Penrose and Martin 1997).

No evidence was found from the field trials that day length changes from delayed seeding date caused any consistent effect on maturity among the substitution lines. This result is consistent with the possibility that the day length requirements for heading are fully met for all Canadian wheat cultivars, and the chromosome substitution lines, by the long photoperiod present from the early to late seeding date in this particular location, as previously mentioned. In generalized terms, such a hypothesis suggests that the day length itself, and the genetic complement for photoperiod genes, is not a large factor in determining differences in heading and maturity for the chromosome substitution lines for the study period. The chromosome 5B substitution line plays an important role through affecting a faster development rate with respect to heading and maturity. Evidence on the possible influence of a higher temperature requiring process for reproductive development in long day plants has been reported in wheat (Gott 1961), which agrees with present findings of later seeding resulting in early heading. Thus, different temperature requirements exist between substitution lines for processes that determine the rate of early development in wheat.

In the present study, the *Vrn* gene system showed that the presence of dominant gene(s) (except CR5A) affected earliness to some extent, under long photoperiods.

Rawson et al. (1998) reported one interesting finding that Dollarbird (spring cultivar), which is considered to have no response to vernalization in the field, seemed to respond to temperatures below 19°C by heading earlier in terms of thermal time. In the present study, CR5A, a winter type carrying all recessive *vrn* genes, showed heading in 1998, where the average temperature from April to July was 14°C. On the other hand it failed to head in 1996, and in 1997 it headed in two seeding dates where the average temperature was 10-11°C respectively. It headed in and between the ranges of usual planting dates, which raises the question about the value for the threshold vernalization temperature in spring wheat. It has been reported that even 12°C-19°C could be rated as a vernalizing temperature range (Rawson et al. 1998). The present findings suggest that there could be some unexplainable effects of vernalization and confirms the published findings that vernalization could occur at higher temperatures. Nevertheless, variability in earliness exists within the sets of chromosome substitution lines that having the same *Vrn* gene and such differences could be due to the differences in sensitivity to growing temperatures and/or light intensity (Stelmakh 1998).

Interaction between the seeding dates and the substitution lines for the three studied environmental variables (GDD, PTU, and PF) also did show an effect for 5A and 5D. However, in 5B the observed result indicated significant interaction in terms of heading in all three years studied. The possible variation in climatic conditions over the three years could account for the observed differences during the study period. The influence of seeding date on environmental variables due to climatic conditions has been reported in spring wheat (Spaner et al. 1999). In the present case the effects of the seeding date on substitution lines show that contrasting differences exist between the studied lines and they are quite different from Cadet and Rescue cultivars.

In the growth chamber the check cultivars and the chromosome substitution lines required considerably more heat units for heading and maturity (Table 4.22), compared to the field (Fig. 4.1, 4.2, 4.3 and 4.7). Changes in day length, temperature, and variation in light intensity, a common occurrence under field conditions, did not occur in the controlled environment. Therefore, the plants were possibly lacking the environmental cues for a developmental switch, since there was only minor environmental variation

under the controlled conditions in the growth chamber. There was little scope for studying the influence of intensity of radiation, as the maximum intensity attainable in the growth rooms was far less than that outdoors in daylight at any time during the natural growing season (Thorne et al. 1968).

Introduction of the substituted group 5 chromosomes (5A, 5B, and 5D) in Rescue or Cadet background showed different *Vrn* gene(s) effects, which conformed to other findings in the literature. The quantitative effects of introducing a chromosome that carries additional dominant *Vrn* genes fulfills the expected results by showing the effects in the direction of early maturity. This two replicate experiment in the field for all years has a relative higher coefficient of variation associated with its protocol so many observed large effects were not significant. More significant effects were found in 1998 than in 1997, and it is possible that higher temperatures in the spring of 1998 influenced maturity differences through vernalization effects, possibly overshadowing control of maturity that might be under the influence of other genetically controlled mechanisms. In both years, however the greatest number of significant substitution effects were found in 5B, and these were of the largest magnitude.

The GDD, PTU and PF heat units has been reported previously as useful environmental variables to calculate and predict the various aspects of wheat phenological development, but prior to this study none of the published literature dealt with maturity and heading to maturity. The present results are more consistent for heading in all the variables, but maturity and heading to maturity showed inconsistent trends during the study period. Another important finding is that all the substitution lines varied widely in the amount of heat unit requirement caused by long day length and temperature to arrive at early heading stage. The present finding could provide some insights into spring wheat heading and maturity as both long day length and temperature contributed as main factors in developmental control.

In the present study the substituted line with 5B (carrying *Vrn4*) showed the greatest reduction in GDD, PTU or PF requirement for heading, and in some cases for maturity traits, as opposed to 5A (carrying *Vrn1*) and 5D (carrying *Vrn3*), that was found

to be consistent over the years in the field study. In many cases, the 5D effects were small to insignificant. The present study indicated that the *Vrn4* genes in the substituted lines showed the most reduced heat unit and accumulated PF requirements, to head and mature early, compared to *Vrn1* and *Vrn3*, which agrees with Driscoll and Jensen (1964).

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Chapter 5. Molecular characterization of 5D substituted lines

5.1. Introduction

The assessment of polymorphism exhibited by molecular markers is an arduous but essential task that facilitates the use of molecular tools by breeders and geneticists. Molecular markers can be used in breeding programs as a criterion for choosing parents, and as a powerful way to follow chromosomal segments carrying genes of interest in a segregating populations (Enjalbert et al. 1999), genetic mapping of traits (Reiter et al. 1992, Sarma et al. 2000), gene cloning (Chong et al. 1998), and marker assisted selection (Michelmore et al. 1991).

Some of the earlier phenotypic markers relied on variation in expressed proteins, termed isozymes (Stuber 1992). Variation in endosperm protein composition (Dexter and Mastuo 1977) and protein components are coded by single co-dominant genes (Mecham et al. 1978) that have enabled scientists to relate protein components to single genes, thus merging the work of classical and molecular genetics. Wheat gluten proteins have been studied at the molecular level due to their abundant transcription levels and highly regulated pattern of expression (Shewry et al. 1997) and are used as markers in cultivar identification. The use of protein electrophoresis to characterize wheat cultivars is favored over the use of morphological characters since these markers are not affected by environmental conditions (Cooke 1984). Also the use of half -seed technology to analyze protein is used in assessing cultivar validation (Lawrence and Shepherd 1980; Zhen and Mares 1992) through non-destructive sampling since the left-over embryonal half is subsequently grown into a plant.

Compared to isozymes, DNA markers are more reliable as they are phenotypically neutral and are not affected by environmental interactions (Lander and Botstein 1989). Useful molecular markers are not usually the gene of interest, but are closely linked with the trait (Weeden 1993).

Restriction Fragment Length Polymorphisms (RFLP's) are genomic molecular markers (Lagercrantz and Lydiat 1996), which detect differences due to nucleotide substitution, insertion, deletion or rearrangement of the genomic DNA sequence (Chang and Meyerowitz 1991). RFLP's behave in a codominant manner, which enables

determination of genotypes in a plant derived from any two parents (Lander and Botstein 1989). However, this technique is expensive, technically demanding, and requires the use of radionucleides, which limit its utility.

The use of Random Amplified Polymorphic DNA (RAPD's) has proven effective in molecular marker application to plant breeding. In this case, arbitrary nucleotide sequences are used as primers in PCR reactions to reveal polymorphisms. These markers behave as dominant traits (a single fragment is either amplified or not at one locus) and segregate in Mendelian fashion (Williams et al. 1990). This technique has been successfully used for genome mapping (Williams et al. 1990; Sewell et al. 1999; Joobeur et al. 2000), and gene identification (Paran and Michelmore 1993). A RAPD-based marker linked to common bunt Bt-10 resistant gene was identified in wheat by Demeke et al. (1996), which later led to the development of a PCR based marker for its identification (Laroche et al. 2000). Amplification Fragment Length Polymorphism (AFLP) is another recent technique for DNA fingerprinting (Vos et al. 1995) that has wide application for DNA analysis. This method does not suffer from reproducibility problems as RAPD's, and does not require previous sequence information like microsatellites.

Bulk Segregant Analysis (BSA) (Michelmore et al. 1991) is a method of detecting DNA markers that are tightly linked to alleles controlling important agronomic traits. This method is designed to locate DNA segments adjacent to genes of interest using sequence specific DNA fragments. This method relies on the development of two bulks of DNA, differing only with respect to a particular trait. The two bulks can be created from any population allowing for all the members of a bulk to be similar for that region under study, but different at all other regions in the genome. This region of the genetic locus is composed of (or contains) a gene(s) of interest and this could be identified by virtue of their presence or absence in the two contrasting bulks.

BSA from F₂ progeny of two crosses segregating for *Vrn3* (5D) in either Rescue (Rescue, *Vrn3*, 4 x RC5D, *Vrn4*) or Cadet (CR5D, *Vrn1*, 3 x Cadet, *Vrn1*) background, were used in the current study in an attempt to correlate phenotypic expression with

genetic polymorphism. The present study also used another genetic stock previously characterized for *Vrn* genes, the near isogenic Triple Dirk (TD) lines (Pugsley 1972).

In the present study, an attempt was made to find RAPD markers linked to the early and late classes of the segregating progeny and in the TD lines. The latter was undertaken with an aim of looking for RAPD bands linked to specific *Vrn* gene(s), since the genotype of these near isogenic lines was known. Since these isogenic lines carry different combinations of known *Vrn* genes, they were used to determine the consistency of finding *Vrn* genes in different wheat backgrounds, which later could be related to the *Vrn* genes substituted in Canadian bread wheat. To minimize the effects of variation in genetic backgrounds, both isogenic materials and progeny of crosses between single chromosome substitution lines were used.

The objective of the present study was, (1) validation of F_1 's by protein analysis, (2) development of the segregating populations from the two crosses segregating for *Vrn3* in either Rescue or Cadet background, (3) phenotyping of F_2 's in growth chambers, (4) to search for polymorphic DNA band(s) co-segregating with the early or late trait for maturity in the segregating F_2 population, and (5) to find polymorphic DNA marker(s) in Triple Dirk-isogenic lines linked to specific *Vrn* gene(s).

5.2. Materials and Methods

5.2.1. Genetic materials :

The chromosome substitution lines used were either in Cadet (CR5A, CR5B and CR5D) or Rescue (RC5A, RC5B and RC5D) background, kindly provided by Dr. André Laroche (Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta) through Dr. K. G. Briggs, (Personal communication). The original crosses of the substituted lines were carried out at Agriculture and Agri-Food Canada Research Centre in Lethbridge, and at the University of Alberta. The genetic constitution of the seed stocks used in this study were as follows: Cadet (*Vrn1*); Rescue (*Vrn3*, 4); CR5A (*vrn*; winter type); CR5B (*Vrn1*, 4); CR5D (*Vrn1*, 3); RC5A (*Vrn1*, 3, 4); RC5B (*Vrn3*); and RC5D (*Vrn4*). In the coded lines the first letter indicates the recipient cultivar and the

three others (letters and number) indicate the source of the specific substituted chromosome.

The present study employed additional genetic stocks, near isogenic TD previously characterized by Pugsley (1972) for *Vrn* genes in an unrelated spring wheat germplasm, obtained from Australia. The following TD lines were used (TD-a, unknown; TD-b, *Vrn2*; TD-c, *vrn*; TD-d, *Vrn1*; TD-e, *Vrn3*; and TD-f *Vrn4*) for RAPD analysis.

5.2.2. Hybridization :

Hybridization was performed between Rescue and RC5D, and between CR5D and Cadet using standard crossing techniques (Poehlman and Sleper, 1995) in growth chambers. The crosses segregating for *Vrn3* were in either Rescue (Rescue, *Vrn3*, 4 x RC5D, *Vrn4*) or Cadet (CR5D, *Vrn1*, 3 x Cadet, *Vrn1*) background. Ten seeds from each of the resulting F₁'s were grown individually in 12.5 cm plastic pots containing MetroMix® in growth chambers (15°C day/10°C night, 16 hr photoperiod). The light intensity at plant level was 350 $\mu\text{mol m}^{-2}\text{s}^{-1}$ from high output fluorescent lights. The plants were watered daily and fertilized twice weekly with Peters 20-20-20™ containing micronutrients. Data were collected for days required to heading, and days to maturity. The seeds from individual F₁'s were collected separately, dehusked and 200 seeds each from two F₁ (one for each cross) were planted under identical conditions to obtain the heading and maturity day requirement data for the F₂'s. Leaf samples from the F₁'s, F₂'s and their parents were collected at the 4-leaf stage, quick frozen in liquid nitrogen, stored at -80°C until lyophilized. The freeze-dried leaf samples were used for extraction of DNA according to Doyle and Doyle (1990).

5.2.3. Protein electrophoresis :

Gel electrophoresis of two main protein fractions (gliadin and glutenin) was performed to verify the parents and F₁'s. The protein extraction and electrophoresis procedure closely followed that described by Kipligat (1995). The quantitation of the extracted proteins was carried out using the BCA protein assay method (Pierce Chemical, Rockford, IL). Half seeds of the parents and F₁'s were ground to a fine

powder in 1.5 ml microfuge tubes using liquid nitrogen and plastic pestles. The gliadins (alcohol soluble fraction) were extracted twice from the flour using 80 μ l 70% ethanol, and pooling the extract. The pellet was air dried and saved for extracting glutenin. The gliadins were separated on 6% acid-PAGE for 3 hr at 80 volts and stained with Coomassie brilliant blue R250.

The glutenins were extracted with 100 μ l TrisCl (pH 6.8) containing 2% SDS by boiling for 3 min in a water bath. The mixture was vortexed, cooled, and the supernatant collected for electrophoretic separation according to Laemmli (1970) on 12% SDS-PAGE at 50 mA for 3 hr. The gels were stained with either Coomassie brilliant blue R250 or ammoniacal silver nitrate.

5.2.4. DNA extraction and RAPD analysis :

Based on the days required for heading in the growth chambers, the F_2 's were grouped into early-, intermediate- and late- flowering classes. DNA from leaf samples of these individuals under those classes was isolated individually and then pooled according to their phenotypic classes for Bulk Segregant Analysis (BSA).

The freeze-dried leaf samples were used for extraction of DNA according to Doyle and Doyle (1990). DNA was quantified by ultra-violet (UV) spectroscopy using GeneQuant™ and by agarose gel electrophoresis against a known standard. The amount of DNA was adjusted to 5 ng/ μ l, and 2 μ l (10 ng) was used in each 20 μ l PCR reaction for Random Amplified Polymorphic DNA (RAPD) analysis using random decamers from the University of British Columbia. DNA samples obtained through two separate extractions were used in the course of the present study.

Each PCR reaction contained 2.5 mM $MgCl_2$, 1x PCR buffer (50 mM KCl + 10 mM Tris-Cl, pH 8.3), 10 ng template DNA, 0.2 μ M of primer and 250 μ M of each dNTP and 1 unit of Amplitaq® DNA Polymerase in a 20 μ l volume. The PCR was carried out in 0.2 ml tubes using GeneAmp 9600™ thermal cycler (Perkin-Elmer Corp). The PCR cycling parameters were, initial denaturation at 94°C for 2 min, 45 cycles of denaturation (92°C) for 30 sec, primer annealing (37°C) for 30 sec, and extension (72°C) for 1 min, followed by a final extension (72°C) of 6 min. After thermal cycling, the PCR products

were mixed with 2 µl of loading dye and separated on 1.3% horizontal agarose gels containing 0.5 µg/ml ethidium bromide for 90 min at 120 volts. The amplified products were visualized under UV transillumination and their pictures taken with a Kodak® MP-3 land camera using Polaroid® 55 films to obtain a permanent record.

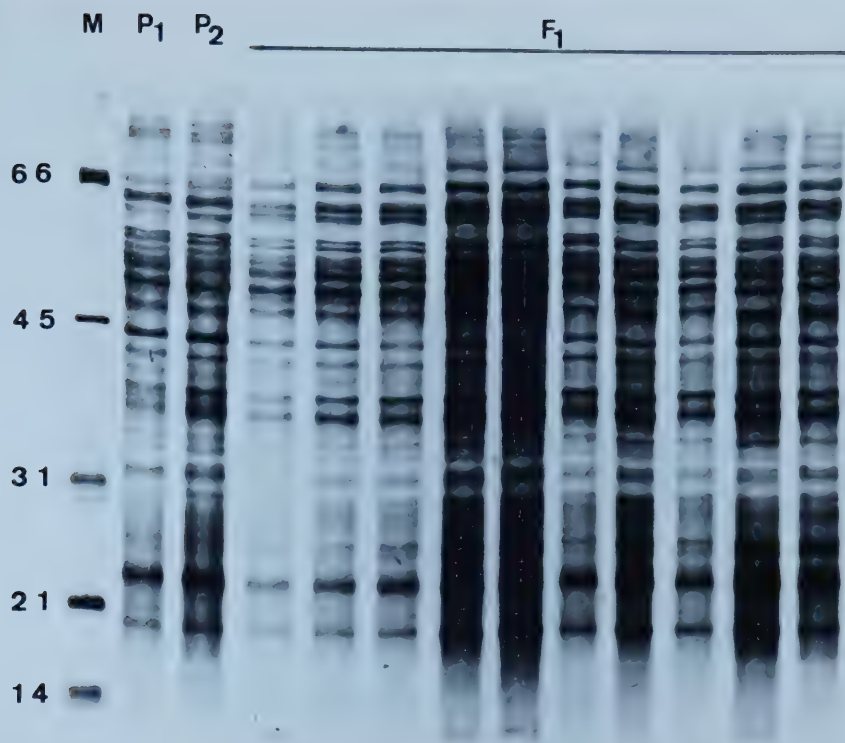
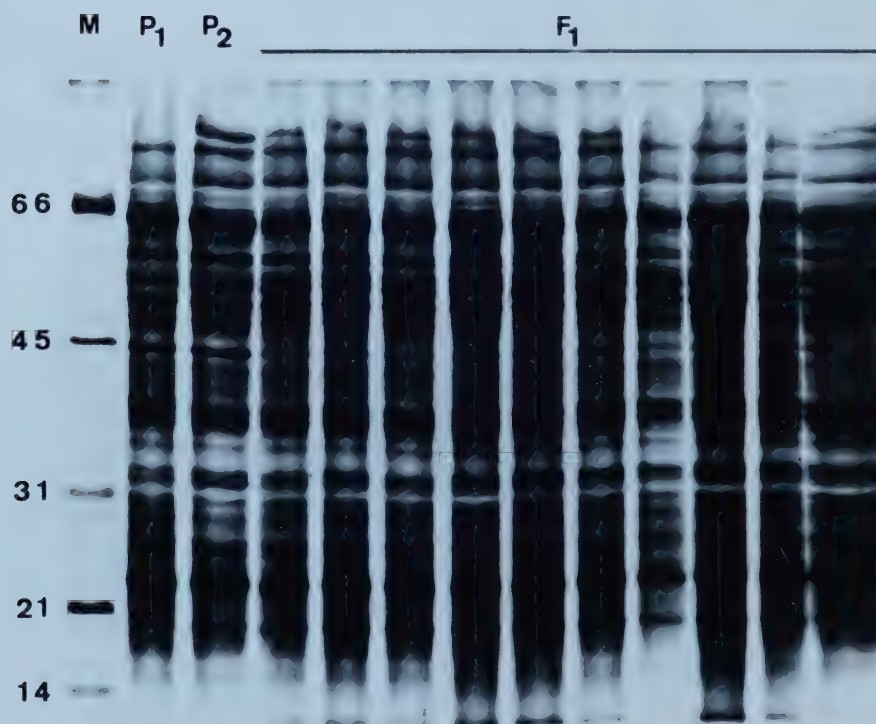
5.3. Results

The electrophoretic separation of gliadin and glutenin did not show any variation among the polypeptide profile of the parents and the F₁'s (Fig. 5.1A, B). The silver stained SDS-PAGE, despite resolving a number of polypeptides, showed the profiles of the individual F₁'s and their parents to be the same in each cross (Fig. 5.1A, 5.1B). However, the protein profile of the parents and their progeny of a given cross were not similar to that of the other cross. A similar result was obtained with acid-PAGE separation of gliadin protein fraction (results not shown).

Growth chamber results for F₂ progenies showed a wide range of requirements in terms of days to heading and days to maturity (Fig. 5.2A, B; 5.3A, B). In the cross involving *Vrn3* in either Rescue (Rescue, *Vrn3*, 4 x RC5D, *Vrn4*) or Cadet (CR5D, *Vrn1*, 3 x Cadet, *Vrn1*) background, the F₂'s required 57–90 days (Fig. 5.2A) and 78–130 days (Fig. 2B) for heading respectively. The parents required 72 and 75 days to head in the former case and 99 and 102 days in the latter (Fig. 5.2A, B). In terms of maturity, the days required were 138–186 days in the former and 151–205 days in the latter cross (Fig. 5.3A, B). The parents in the first cross required 165 and 171 days, and in the second cross required 181 and 184 days to reach maturity. Scatter plotting of the segregating F₂ progeny and their parents under growth room conditions did not reveal any correlation between days to heading and days to maturity in either Rescue x RC5D (Fig. 5.4A) or CR5D x Cadet (Fig. 5.4B).

About 300 random primers from UBC were initially screened with DNA samples from the TD-lines. Twenty-two primers were found to produce polymorphic DNA bands with the above-mentioned DNA samples, out of which 16 were found to be reproducible after three rounds of screening (Fig. 5.5). The results from these primers

Fig. 5.1. Polyacrylamide gel electrophoretic separation of gliadin protein fraction extracted from parental and F₁ wheat seeds of crosses segregating for *Vrn3* were in (A) Rescue (Rescue, P₁, *Vrn3*, 4 x RC5D, P₂, *Vrn4*) or (B) Cadet (CR5D, P₁, *Vrn1*, 3 x Cadet, P₂, *Vrn1*) background. Molecular weight standards (M) are wide range SDS-PAGE markers from BioRad®, showing the size of polypeptides in kda.

A**B**

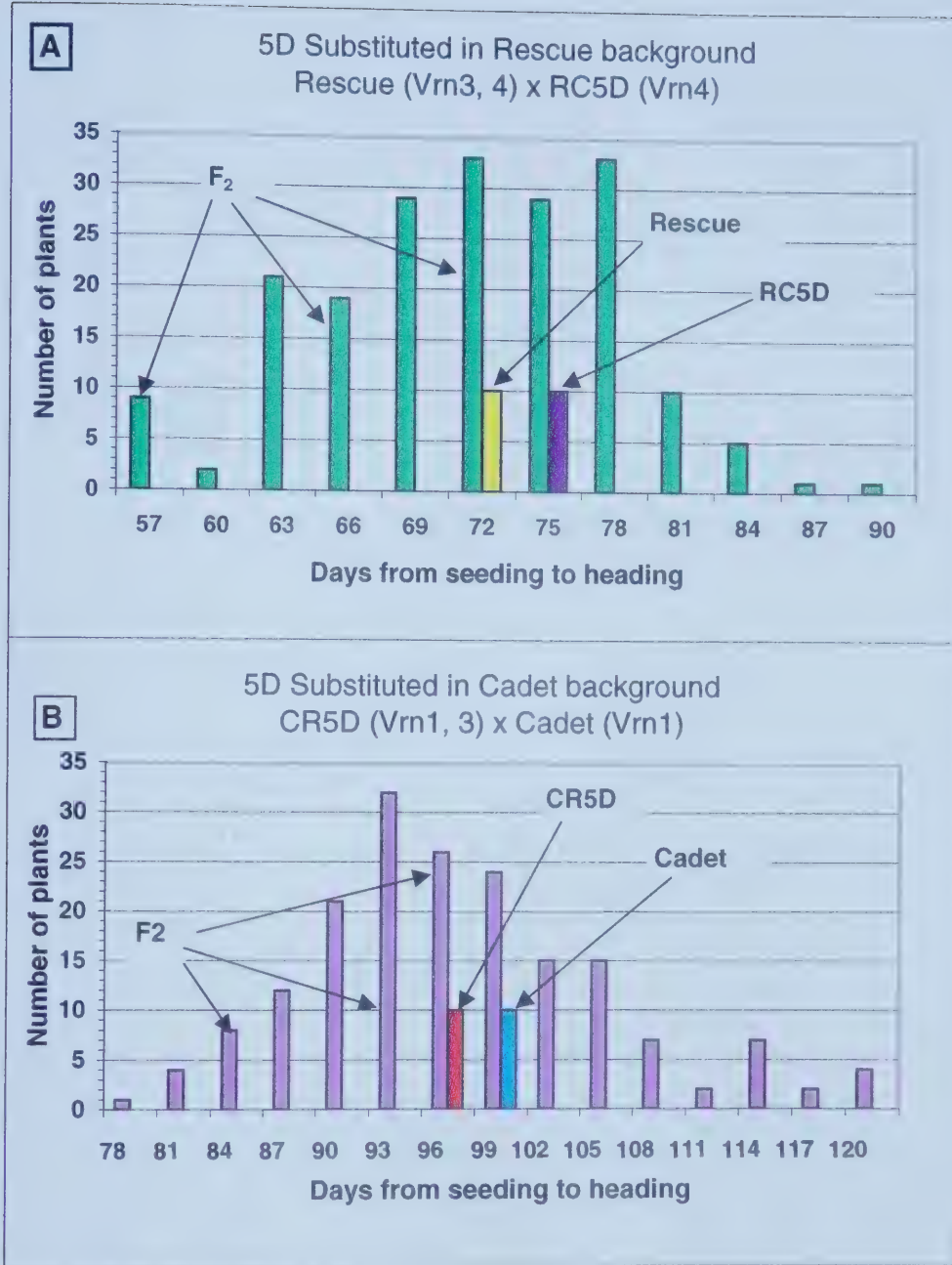


Fig. 5.2. The effect of substitution of chromosome 5D in (A) Rescue and (B) Cadet background on days to heading under long-day, non-vernalized conditions (15°C night/10°C days, 16hr photoperiod) in segregating F₂ populations and genotypes. Standard error values for days to heading are 1.44, 1.55, 2.23 and 1.82 for Rescue, RC5D, CR5D and Cadet respectively.

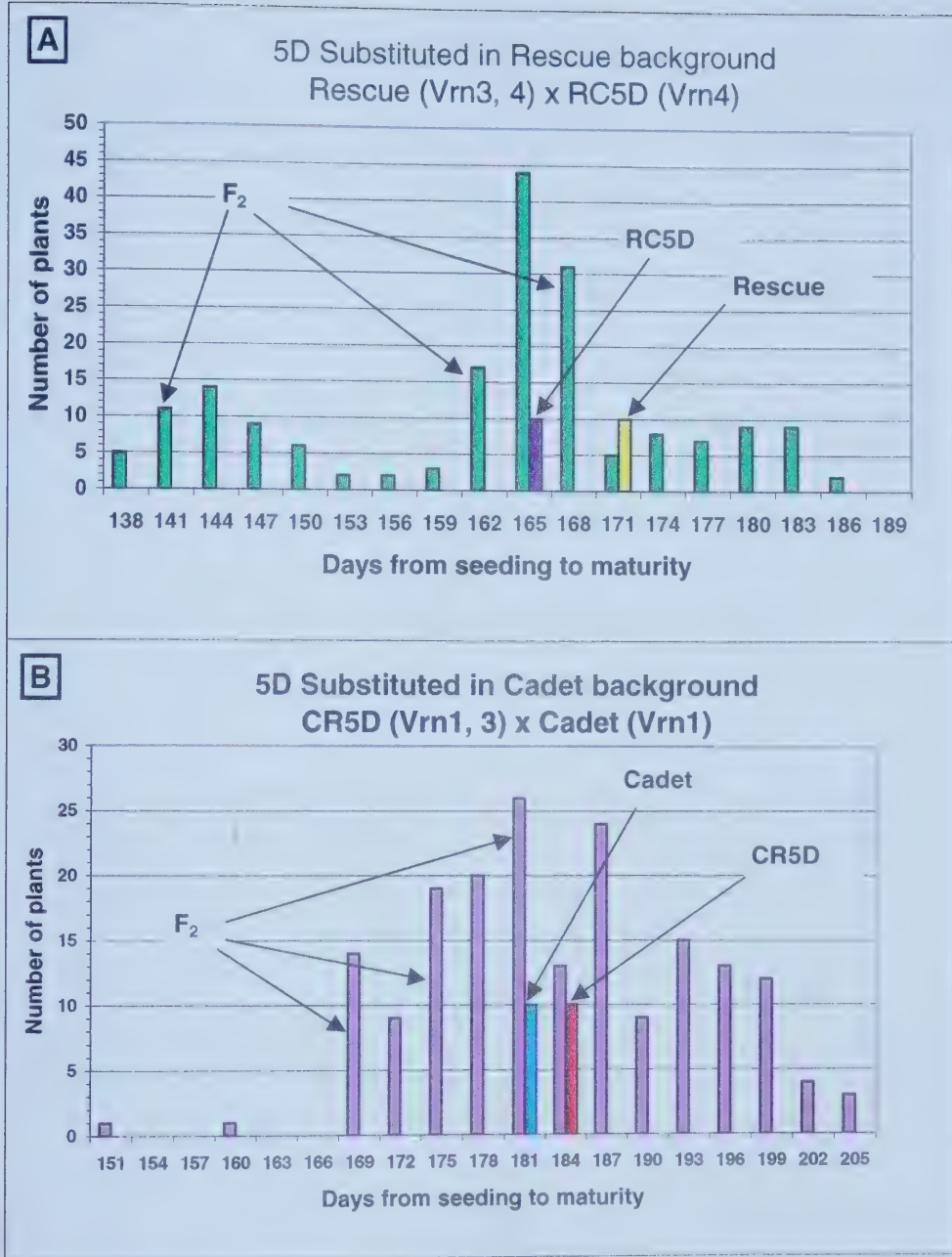


Fig. 5.3. The effect of substitution of chromosome 5D in (A) Rescue and (B) Cadet background on days to maturity under long-day, non-vernalized conditions (15°C night/10°C days, 16hr photoperiod) in segregating F_2 populations and genotypes. Standard error values for days to maturity are 4.60, 5.14, 2.75 and 1.61 for Rescue, RC5D, CR5D and Cadet respectively.

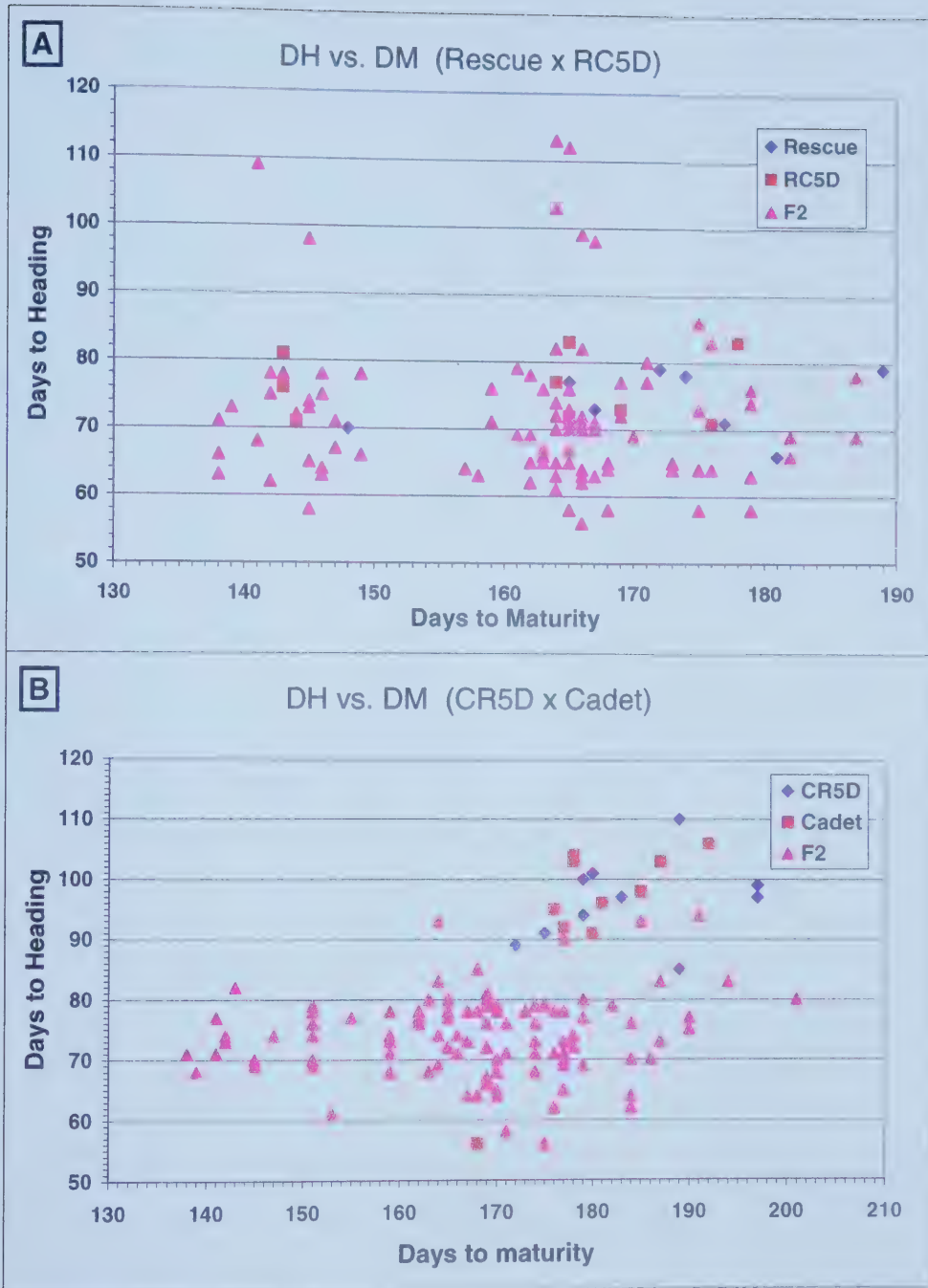
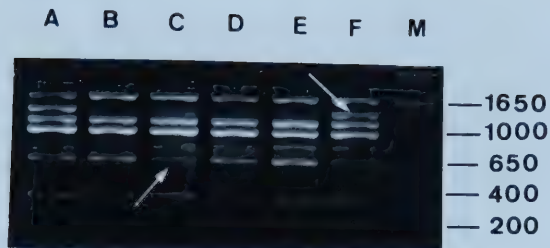


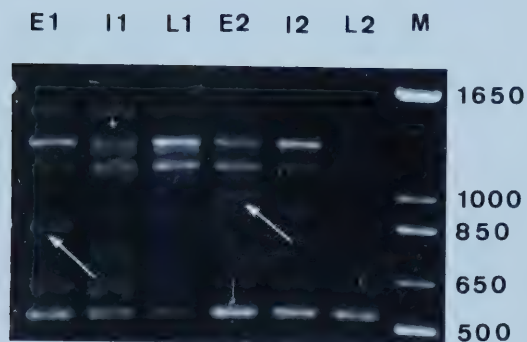
Fig. 5.4. Correlation between days to heading and days to maturity in the parents and the segregating F₂ progeny of crosses between (A) Rescue and RC5D, and (B) CR5D x Cadet in growth rooms at 15°C night/ 10°C days, 16 hr photoperiod.

Fig. 5.5. The Random Amplified Polymorphic DNA (RAPD) profile using DNA extracted from (A) TD lines a to f employing primer UBC332, (B) bulked DNA from segregating F₂ progeny of crosses segregating for *Vrn3* were in either (1) Rescue (Rescue, *Vrn3*, 4 x RC5D, *Vrn4*) or (2) Cadet (CR5D, *Vrn1*, 3 x Cadet, *Vrn1*) background showing Early (E), intermediate (I) and Late (L) bulks employing primer UBC295, and (C) parents, F₁'s and individual F₂'s employing primer UBC295. The molecular weight markers (M) are 100 bp standard from Life Technologies®, showing the fragment size as bases/nucleotides long.

a



b



c

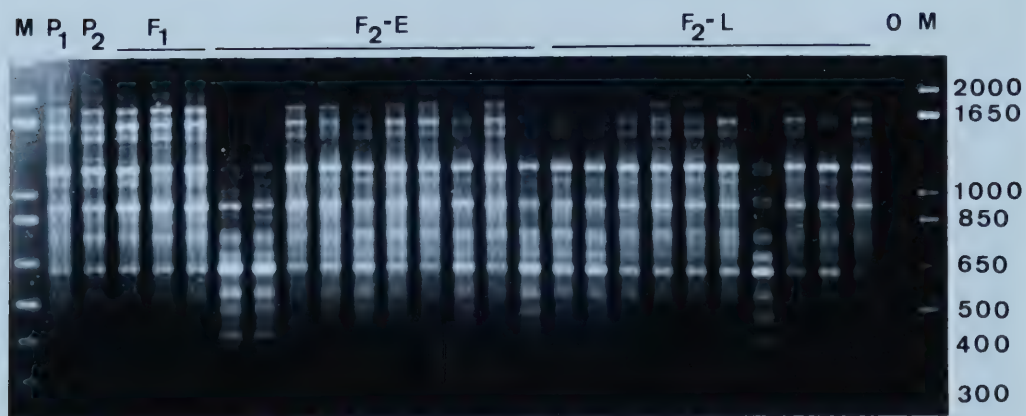


Table 5.1a. Approximate size of unique bands (in base pairs) produced in near isogenic lines of Triple Dirk employing random decamer primers.

Primer	TD-a	TD-b	TD-c	TD-d	TD-e	TD-f
UBC134				600		
UBC 135						1300
UBC 219						1650
UBC 224				2100	1700 400	1600
UBC 237					500	
UBC 248				650		
UBC 250				500	1650	
UBC 253				1000	1650	
UBC 254				1500	2000	850
UBC 256					1600	
UBC 266					1000	
UBC 274					1350	900
UBC 278					1100	900
UBC 285					980	
UBC 332	1500		600			1500
UBC 362		350		350		

Table 5.1b. Approximate size of unique bands (in nucleotides) produced in bulked F₂ DNA samples of Cross 1 (Rescue x RC5D), and Cross 2 (CR5D x Cadet) employing random decamer primers.

Primer	Cross 1		Cross 2	
	Early	Late	Early	Late
UBC 225	670	550	500	
	850	750	600	
	1600		800	
UBC 278		1200	1200 1300	1150
UBC 295	840		1000	
UBC 297	1000	1050	520	1100
	1200		650	
			1000	

are summarized in Table 5.1a. For example, with primer UBC332 a unique fragment of approximately 600 nucleotides was amplified using the DNA from TD-c, while a unique fragment of approximately 1500 bases was amplified using DNA from TD-a and TD-f (Fig. 5.5A). Similarly, unique bands were obtained with some of the individual TD lines with the 15 other primers as shown in Table 5.1a.

The primers showing polymorphism in TD lines and some other primers were used with bulked DNA samples from the late and early- heading genotypes of F_2 from both the crosses. The primers UBC225, UBC278, UBC295 and UBC297 appeared to amplify unique bands with either early- or late- bulked samples (Table 5.1b). For example, with the primer UBC 295, the early bulk in cross 1 produced a unique band of approximately 840 bases, while that of cross 2 amplified an unique band of 1000 bases (Fig. 5.5b). The results, however, were not very consistent, the same primers producing different kinds of banding profiles with DNA from the same samples, although 3-4 repeats of the same experiment produced similar results. Based on the results obtained from the bulked DNA, the individual DNA samples were used to determine their banding patterns with primer UBC295. Also, when the segregation of the probable bands in the individuals of the bulked progeny and their parents was carried out, no consistent pattern was observed (Fig. 5.5c). The parents were not found to contain any unique bands, therefore the appearance of unique bands in the F_1 's and segregation in the F_2 's would not be expected.

5.4. Discussion

The gliadin proteins (alcohol soluble fraction) showed the most polymorphisms and was easily extracted and fractionated (Cooper et al. 1986). However, in the present experiments, no difference was noted among the polypeptide profiles of all the samples including the parents and the F_1 's. This observation could result from the limitation of the staining techniques, especially since Coomassie staining is known to be weakly sensitive. However, the failure to detect polypeptide variation in glutenin fraction among the parents and the F_1 's in the silver-stained gels cannot be readily explained. This is

surprising given the fact that the gels resolved a large number of polypeptides, therefore this issue is not related to their separation or resolution. It could possibly be due to the fact that the crosses were, for example, between Rescue \times RC5D, and CR5D \times Cadet. In this case, the two parents differed by only one chromosome (the 5D coming from the complementary background), therefore the total genetic variation between the two parents might be expected to be limited. It is, therefore, conceivable that the differences in protein profile of these two parents would not have been significant. Indeed, the genes for glutenin and gliadin are reported to reside on long and short arms of chromosome 1 and 6 (Zhen and Mares 1992), therefore the substitution lines are not expected to vary in terms of their storage protein profile. However, despite not observing any differences between the parents and the progeny within a given cross, differences were observed between the parents and the progeny of the different crosses.

For days to heading, and days to maturity in the segregating F_2 population, the individuals exhibited transgressive segregation for earliness and lateness. The F_2 's showed heading and maturity dates beyond both the parental values in both crosses (Fig. 5.2, 5.3). This result suggests that other genes may be present on chromosome 5D, which are also affecting days to heading and days to maturity. There could be other genes affecting developmental rate (i.e. photoperiod on chromosome 2B and 2D, 'earliness *per se*' on 5A and 3A.) involved here, since the dominant *Vrn* genes alone could not account for such segregation.

By using random decamers, some unique DNA fragments were obtained in several TD lines (Fig. 5.5a) that could be related to their *Vrn* gene status. Without further study, this cannot be resolved with certainty, however screening of additional lines and sequencing of the products could resolve this problem. The unique 1500 bp DNA fragment observed in TD-a and TD-f could possibly be characterized in future study to investigate the possible *Vrn* composition of TD-a line which is not known. These primers were used with the bulked DNA from early- and late- heading F_2 individuals in both crosses. Although a promising result was obtained with the BSA (Table 5.1b), when some of these primers were used with the individuals that made up the bulks, and their parents, the result was not consistent (Fig 5.5c). RAPD analysis is known to suffer from

lack of reproducibility (Ellsworth et al. 1993; Riedy et al. 1992), and this could explain the inconsistency in the results obtained. This could be further aggravated by the large genome size and repetitive nature of wheat DNA, and also due to two different independent DNA extractions used over the course of the study. The different DNA extractions could lead to possible variations in the amount of contaminants that could affect the RAPD banding patterns. Also the quantification of DNA following independent extractions at different stages of the study could introduce variations, and DNA quantity is known to affect the RAPD profiles (Ellsworth et al. 1993; Riedy et al. 1992).

Another possible explanation for failure to find molecular markers associated with the earliness trait on chromosome 5D could come from the transgressive segregation observed in the F_2 's, which suggest complex inheritance (QTL) of earliness genes on this chromosome, rather than being controlled by a single dominant gene (i.e. *Vrn3*). The search for a single, genetic marker for earliness, therefore, might not be possible in these crosses, but multiple markers may be required to detect quantitative trait loci (QTL) that could control these characters in Canadian spring wheat. Also, since the parents employed in both the crosses had very similar days to heading and days to maturity requirements (Fig. 5.3, 5.4), this may limit ones ability to obtain molecular markers related to these traits.

Although some unique bands were observed with both TD lines and the bulked DNA from the segregating F_2 , there was no consistency between these two sample sets. For example, a unique band was observed in TD-e (containing *Vrn3*) with the primer UBC278, no such band was present in the bulked F_2 samples. Similarly, several primers (e.g. UBC224, UBC253, UBC254 etc.) produced unique bands in TD-e, but no such bands were present when the bulked DNA samples were used. On the other hand, bulked samples used with primers (UBC225, UBC295, UBC297) produced unique bands, but failed to do so with the TD lines. This could be due to the differences in the genetic background of the samples, and indicates that the search for RAPD markers based on their genetic constitution alone may not be very successful.

As our present study focused mainly on a search for a *Vrn3* marker, due to the availability of the seed stock, future research may be carried on towards search for markers related to chromosome 5B and possible *Vrn4* effects based on the present findings. This is given the fact that the magnitude of variation observed in *Vrn4* effect is much more and is also more consistent compared to *Vrn3* effect. Although RAPD markers are extensively used in linkage studies, they do not require prior sequence information and therefore may suffer from reproducibility problems as have been mentioned previously. The AFLP method could provide more information, is reported to be more reproducible, and could be tried in future studies. As the present finding suggests a multigenic trait, therefore other factors could regulate heading response in the Canada Spring wheat cultivars. Furthermore, search for QTL or multiple markers would be another possibility that could be explored. Future biometrical studies can also attempt to determine the number of genes involved in the segregating F₂ population to determine the number of genes involved resulting in the observed segregation pattern and whether transgressive is taking place.

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Chapter 6. Discussion and conclusions

6.1. Discussion

The potential to grow spring wheat in the northern areas of the Canadian prairies can be increased, however, early maturing genotypes with suitable genetic constitution are necessary to gain insights on the genetic control mechanisms for heading and maturity. The environmental conditions in which wheat genotypes are sown are important in controlling phenological development related to agronomic traits such as time to heading and time to maturity. It is apparent that temperature, vernalization and photoperiod all can significantly alter the time required from seedling emergence to maturity.

With respect to the field characterization of the check cultivars, they exhibited a wide range in days to heading and maturity over all the years studied, pertaining to one of the objectives of the present study. The *Vrn* gene composition in the check cultivars is not available in the published literature but the ranking of the cultivars in respect to earliness agrees with a published report (DePauw et al. 1995). The series of 5B substitution lines studied did cover a similar maturity range as that found in the Canadian check cultivars selected. Therefore, the Cadet/Rescue chromosome substitution system was potentially suitable to explain the full range of heading and maturity differences in relation to days required for the phenological development.

In terms of the effect of chromosome substitution, the 5B substituted lines played an important role through affecting a faster development rate by reducing the time necessary for heading and maturity compared to lines containing 5A and 5D. CR5A headed in most of the seeding dates in 1998, although it is a winter type and carries all recessive genes, presumably through vernalization in the field at higher temperature. The relative ranking of the chromosome substitution lines from seeding to heading were consistent over the study period, and delayed seeding dates resulted in early heading when scored as days elapsed.

The quantitative effect of introducing a chromosome that carries additional

dominant *Vrn* gene(s) fulfils the expected results by showing the most effect in the direction of early maturity. The most significant results were found in 1998 rather than in 1997, and it is possible that higher temperatures in the spring of 1998, influenced maturity differences through vernalization effects, possibly overshadowing control of maturity that might be under the influence of other genetically controlled mechanism(s). In both Rescue and Cadet background the presence of *Vrn4* had the largest effect in reducing the time to heading, followed by *Vrn1*, and finally, *Vrn3*.

In the growth chamber, the check cultivars and the substitution lines required considerably more days for heading and maturity compared as to the field. This is more due to the cooler prevailing temperature in the growth chambers (15°C days/10°C nights). Also the lack of environmental variation may not have provided the plants with the necessary cues to developmental switch. The relative rankings of the cultivars and the chromosome substitution lines, however, remained the same as that observed with the field study. The parents of the crosses employed for obtaining segregating populations, when grown in growth rooms showed discrepancy in relative days required for heading and maturity (Fig. 5.2 and 5.3). However, the differences between these genotypes were very slight, and if the standard errors of the samples are taken into account, the observed variations are not significant.

The field experiments examined the days to heading of the group 5 chromosome substituted lines, which seemed to be affected by different seeding dates, but maturity, and heading to maturity did not show any consistent trend. In the field trial, over a 3 year period, day-length changes from early to late seeding date did not show any significant interactive effect on the relative heading and maturity requirements of the substitution lines or the check cultivars. Thus day-length itself, and the genetics of the photoperiod genes, was not a major factor in determining the differences for heading and maturity for the chromosome substitution lines during the study period.

In terms of the environmental components studied, the check cultivars exhibited a wide range of GDD, PTU and PF requirement throughout the years studied for heading and maturity. Late seeding dates produced early heading with a concomitant reduction of GDD requirement, as was observed when scored as days elapsed. The field study reveals

the fact that accumulation of GDD, PTU, and PF for heading and maturity traits of check cultivars was also in the same range as that of the substitution lines, again reinforcing the validity of using substitution lines. Possibly, this also indicates that the cultivars may contain similar *Vrn* genes or combinations thereof.

As indicated previously, days required is the simplest form of criteria that can be used to measure heading and maturity requirements of wheat cultivars, and has been used extensively in literature. In the current study an attempt was made to determine which component of the environmental variable (i.e. temperature, photoperiod, photon flux) is more appropriate in understanding genetic control of heading and maturity. Overall, when the data were analyzed in terms of days, the check cultivars and the chromosome substitution lines tended to head earlier due to later sowing dates. However, when the data was analyzed in terms of GDD, sowing date did not appear to have a major effect, suggesting that the accumulated heat unit must be a critical factor in controlling heading. Very similar trends were obtained when the data was evaluated in terms of GDD and PTU, as indicated previously. Since these two parameters differ only with respect to photoperiod, we can possibly rule out the effect of this variable in controlling heading and maturity. The available photoperiod at this northerly latitude may well be beyond the critical requirement, therefore increased photoperiod with later seeding dates did not elicit any response. In terms of photon flux requirement, the check cultivars and the chromosome substitution lines formed heads earlier in later sowing dates, indicating this variable of environmental component affects heading of spring wheat. However, in the absence of detailed and systematic analysis, it is not possible to ascertain which of the two environmental variables (temperature or photon flux), is the driving force behind head formation in spring wheat genotypes. On the other hand, it is conceivable that both of them may contribute to head formation.

Interaction between seeding dates and substitution lines for days to heading, maturity and environmental variables (GDD, PTU and PF) did show some genetic effect of substituting chromosome 5A and 5D, but 5B produced the most significant interaction in heading trait in all three years. The substitution of 5B carrying the *Vrn4* gene resulted in the highest reduction in number of days required for heading, along with reduced heat unit requirement, compared to 5A carrying *Vrn1*, and 5D carrying *Vrn3*.

Data on days to maturity and heading to maturity for spring wheat were not widely available in the published literature prior to this study. The results obtained are more consistent for heading (in days and all the other variables GDD, PTU and PF used in this study) but maturity showed inconsistent trends during the 2-year study period. The trend was even more inconsistent in terms of heading to maturity, presumably due to the additive variation in these two variables. In the present study, 5B (carrying *Vrn4*) showed the most reduced GDD, PTU and PF requirement for heading and maturity compared to 5A (carrying *Vrn1*) and 5D (carrying *Vrn3*), which was found to be consistent over the 3 year study period.

The present study also reported at the segregation of chromosome 5D substitution lines for *Vrn3* gene in F₂ for days to heading and days to maturity. Segregation data of the F₂ individuals exhibited transgressive segregation for earliness and lateness, showing heading and maturity dates beyond parental values in both crosses. This suggests that other genes may be present on chromosome 5D that are affecting days to heading and days to maturity, since dominant *Vrn* genes alone could not account for such a wide range of segregation in the F₂.

The storage protein analysis of the gliadin and glutenin proteins failed to show differences in the polypeptide profiles of all the samples including the parents and the F₁'s. This is possibly not related to the separation or resolution of the polypeptides. It could possibly be due to the fact that in the crosses the two parents differed by only one chromosome (the 5D coming from the complementary background), therefore the total genetic variation between the two parents might be expected to be limited. It is, therefore, conceivable that the differences in protein profile of these two parents would not have been significant. Indeed, the genes for glutenin and gliadin are reported to reside on long and short arms of chromosome 1 and 6 (Zhen and Mares 1992), therefore the substitution lines are not expected to vary in terms of their storage protein profile. However, the parents and the progeny of the two crosses had different polypeptide profiles, indicating the positive resolving power of this technique.

RAPD analysis with random decamers produced unique DNA fragments in the near isogenic TD-lines that could possibly be related to the *Vrn* genes known to be

present in those lines (i. e. *Vrn3* in TD-e). Further screening and sequencing of these products in the future could possibly provide the identity of those unique DNA fragments. The primers that produced unique bands in the TD-lines were used with the bulk DNA for early- and late- heading classes of F₂ individuals in both crosses segregating for *Vrn3*. Unique RAPD fragments were obtained when bulked DNA was screened, but unfortunately no such bands were consistently reproduced when the individual DNA samples making up the bulk were used. This may be due to the large genome size and the repetitive nature of the wheat genome as mentioned previously. The inconsistency of the results towards finding a molecular marker(s) associated with the earliness trait on chromosome 5D could also come from the transgressive segregation observed in the F₂'s, which suggest complex inheritance (QTL) of earliness gene on this chromosome, rather than control by a single dominant gene (i. e. *Vrn3*). The search for a single genetic marker for earliness, therefore, might not be possible in these crosses, but multiple markers may be required detecting quantitative trait loci that could control these characters in Canadian spring wheat.

McGinnis et al., (1992) opined that improved spring wheat cultivars could be developed for northern areas by reducing the time to maturity by as few as two days, while maintaining the grain yield and protein content. Plant breeders have pursued hybridization of wheat with other grass species with the objective of obtaining lines with superior traits. Introgression of the *Vrn* genes from related species into cultivated wheat offers new possibilities for deliberately altering ear emergence and maturity time. The use of introgressive wheat-rye chromosome substitution lines carrying *Vrn6* and *Vrn7* has also been reported (Stelmakh and Avsenin 1996; Goncharov 1998).

Our present study focused mainly on a search for a marker related to *Vrn3* (5D), due to the availability of the seed stock. However, this line was much later than the other two lines (5A, 5B), and further research in this system would not be very informative. Future research may be carried on towards search for markers related to chromosome 5B and possible *Vrn4* effects based on the present findings. Although RAPD markers are extensively used, they suffer from reproducibility problems, and are not as informative as other markers. The AFLP method may provide more information, is highly reproducible and should be tried in the future. As the present finding suggests a multigenic trait for

Vrn genes probably regulate heading response in the Canadian Spring wheat cultivars. Furthermore, search for QTL or multiple markers would be another possibility that could be explored.

The heat unit requirements as GDD would be a useful environmental variable to study for different locations in the future. Further greenhouse or growth chamber studies related to the present objectives possibly would not provide any meaningful information. Also, the high cost associated with controlled environmental studies would make it unattractive. Field studies using more replicates, field trials in several locations for 2-3 consecutive years, and maintaining similar sowing dates each year, would provide more reliable information on the photoperiod response of substitution lines. Also the data obtained from such experiments will be more statistically sound, and conclusions could be drawn from such data with confidence. However, this would entail a higher cost (i. e. more replicates, several locations etc.), which could possibly be somewhat offset by reducing the number of seedlings to about once a week, and over a shorter time frame. Based on the present information, further study of chromosome 5B substitution lines carrying *Vrn4* genes will possibly be more informative in future, since lines containing this gene were found to produce the most positive results in terms of heading and maturity. In future studies, one can choose either GDD or PTU to determine the heat unit requirements for phenological development of plants, since they were more appropriate descriptors compared to days.

6.2. Literature cited

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Appendix

1

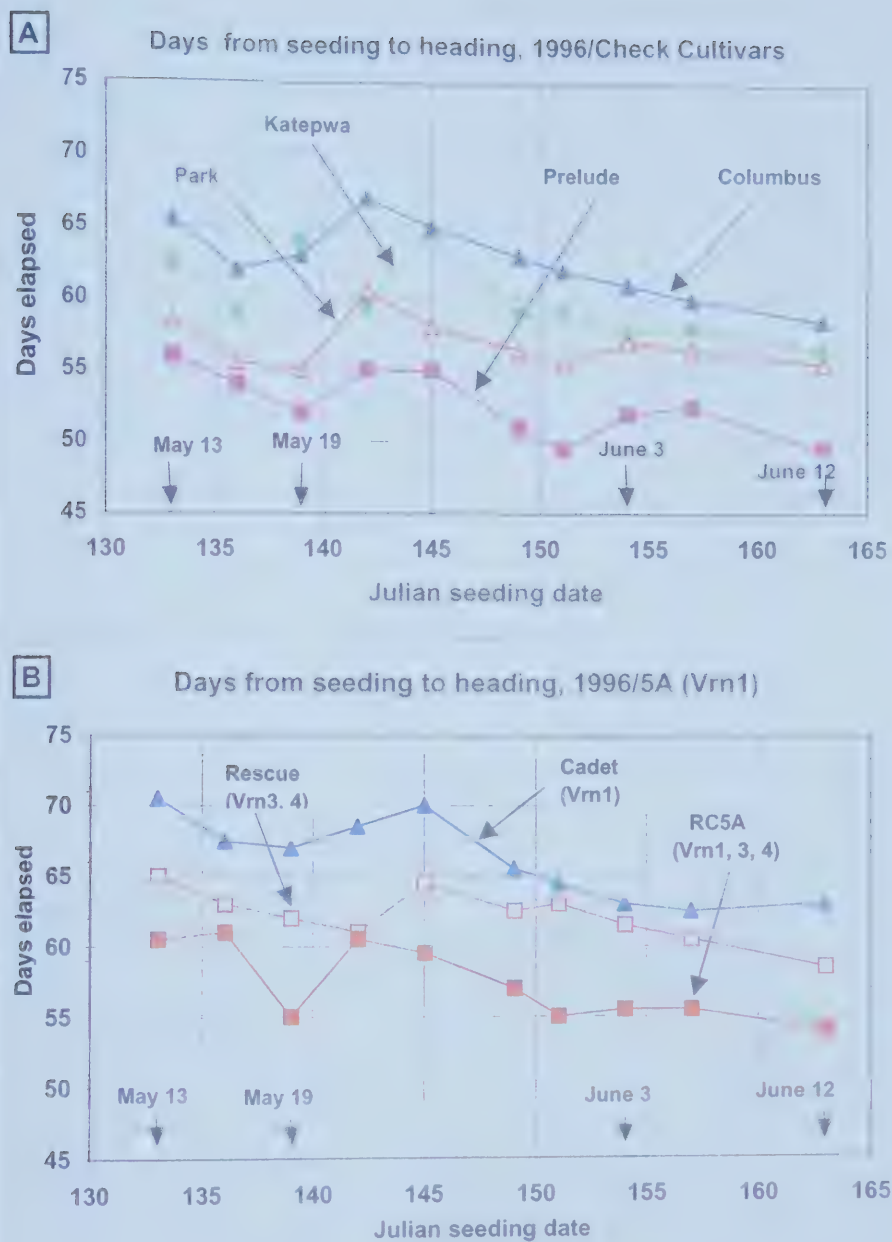


Fig 7.1 The effect of seeding dates on days elapsed from seeding to heading in check cultivars (A) and 5A chromosome substitution lines and their recipient parents (B) in 1996 at Edmonton Research Station.

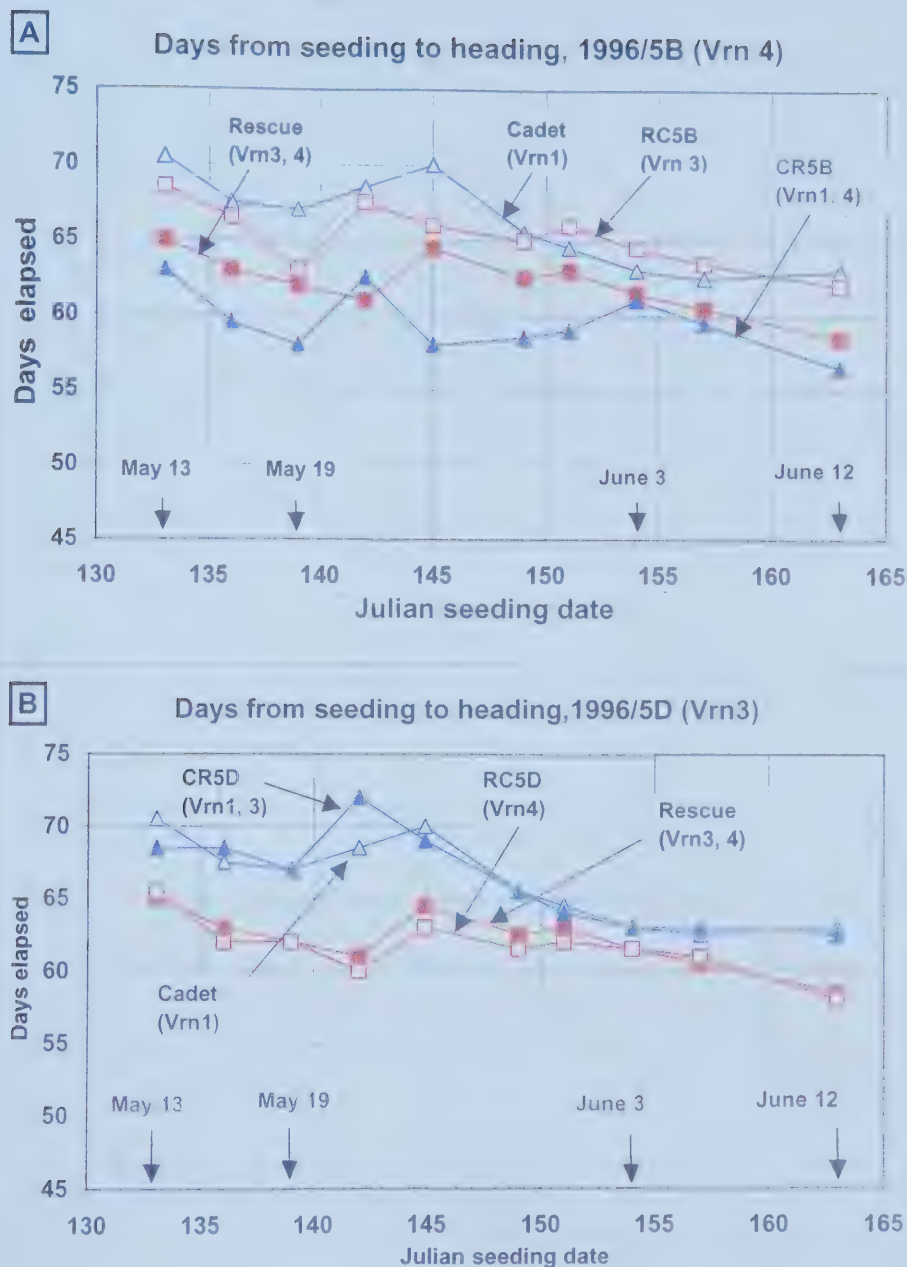


Fig. 7 2. The effect of seeding dates on days elapsed from seeding to heading in 5B (A) and 5D chromosome substitution lines (B) and their recipient parents in 1996 at Edmonton Research Station.

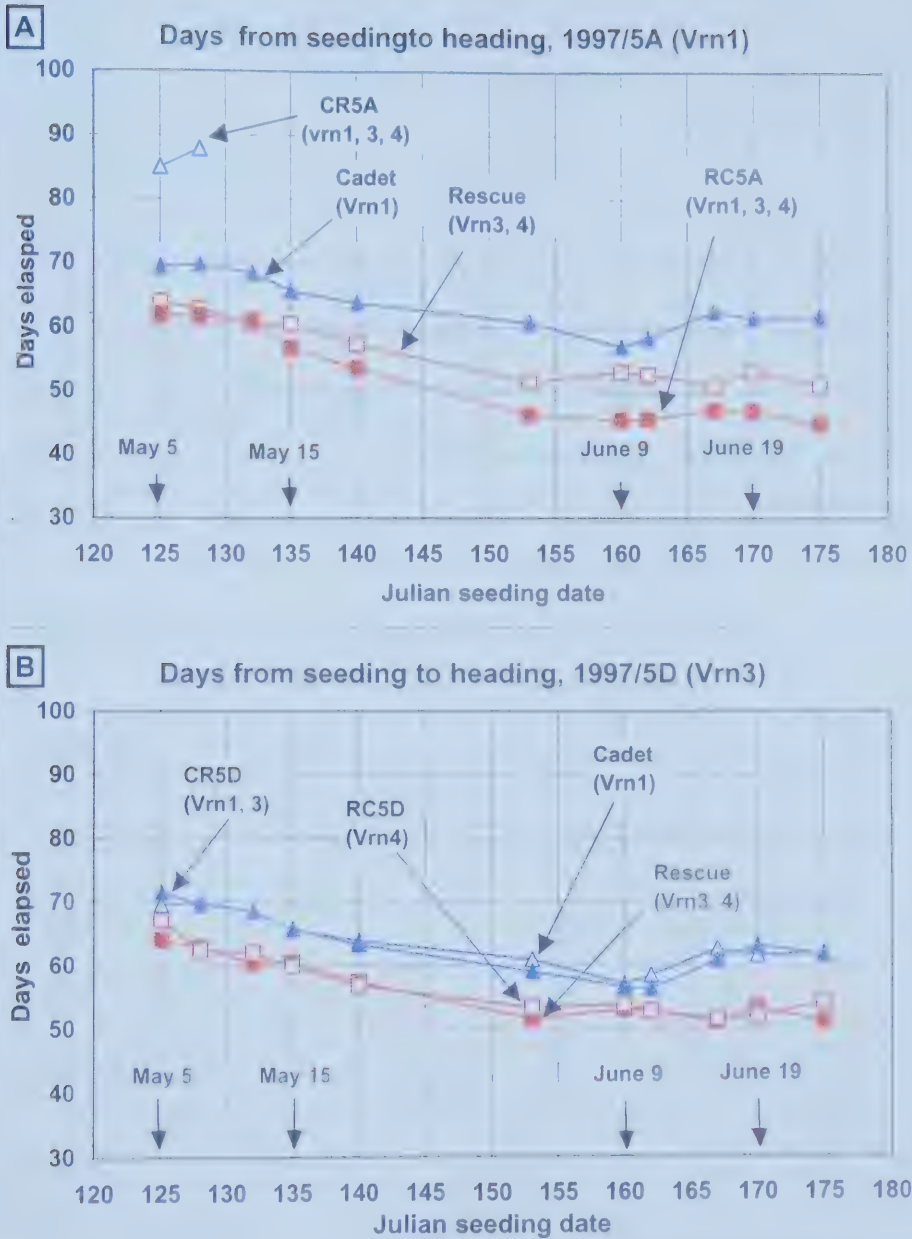


Fig 7.3 The effect of seeding dates on days elapsed from seeding to heading in 5A (A) and 5D chromosome substitution lines (B) and their recipient parents in 1997 at Edmonton Research Station.

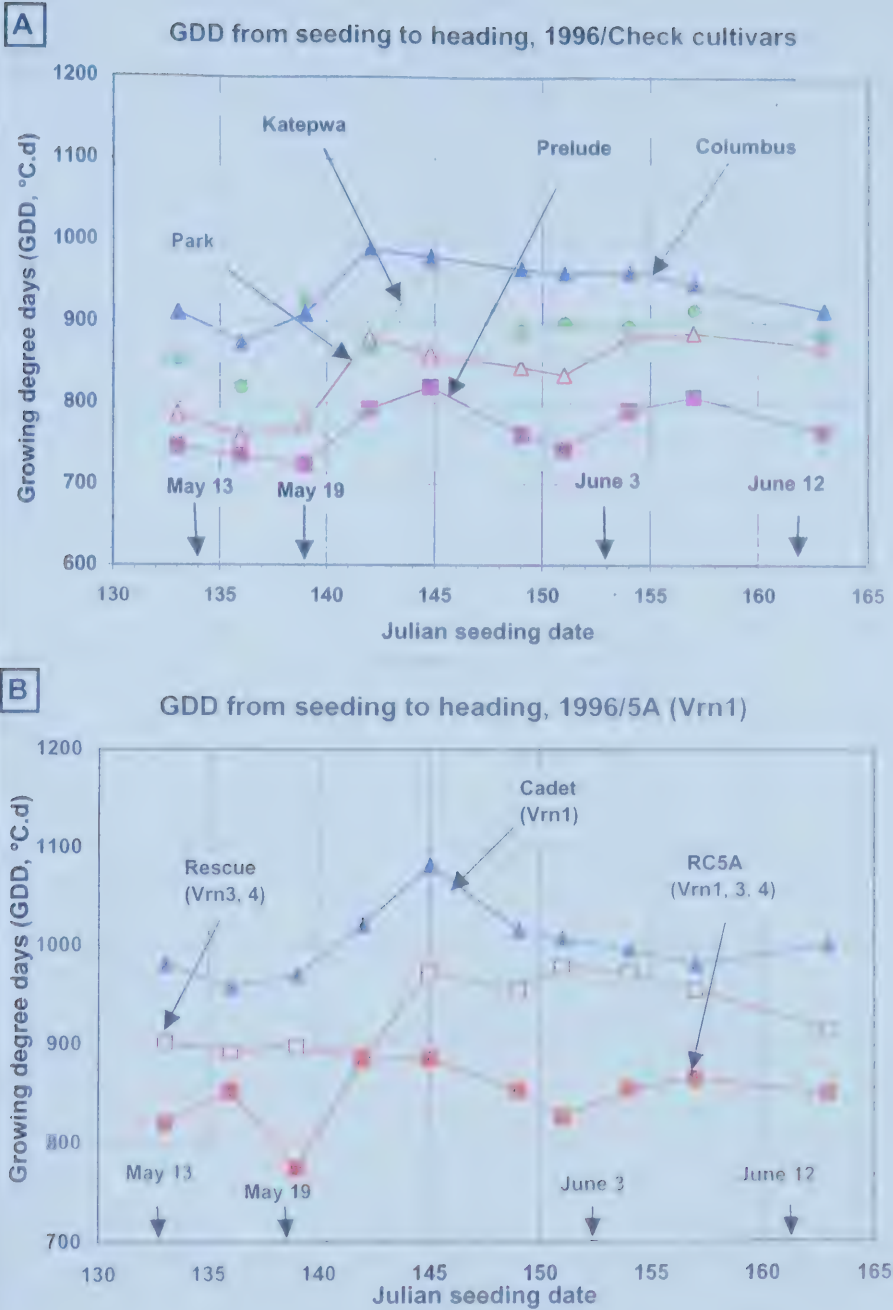


Fig. 7.4 The effect of seeding dates on growing degree-days (GDD) required for heading in check cultivars (A) and 5A substitution lines and their recipient parents (B) in 1996 at Edmonton Research Station.

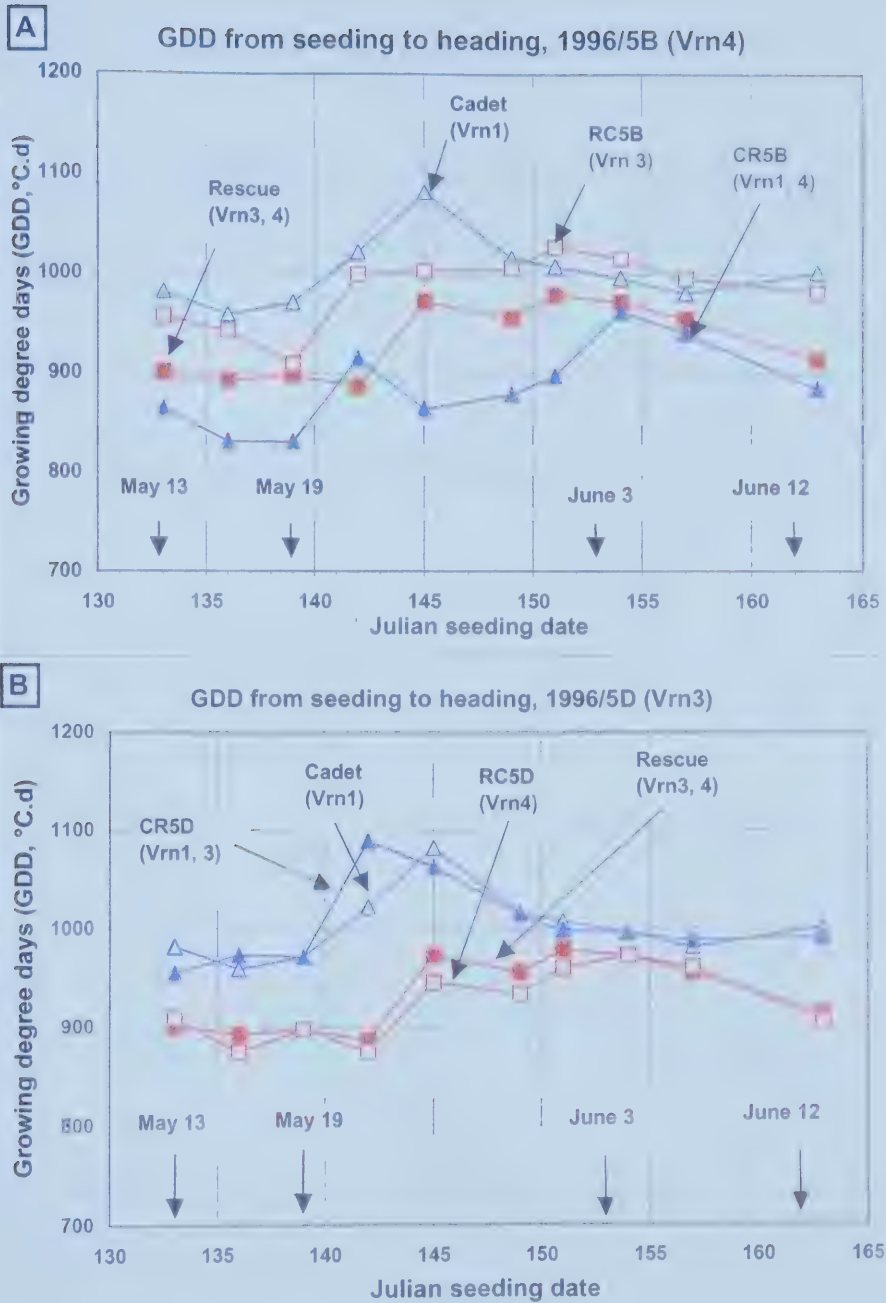


Fig 7.5 The effect of seeding dates on growing degree-days (GDD) required for heading in chromosome 5A (A) and 5D (B) substitution lines and their recipient parents in 1996 at Edmonton Research Station.

Examples of analysis of variance showing interaction between seeding dates and cultivars.

Table 7.1. Analysis of variance comparing Cadet with CR5B substitution line of spring wheat, at Edmonton Research Station in 1996 in days to heading, * and ** indicates significant difference at 5 and 1 percent level respectively.

Source	DF	Mean Square	F Value	Pr > F
rep	1	38.03	19.28	0.0003**
sdate	10	29.70	15.06	<0.0001**
cult	1	442.23	224.21	<0.0001**
sdate x cult	9	8.17	4.14	0.0044**
Error	19	37.48	1.97	
Corrected Total	40			

Table 7.2. Analysis of variance comparing Rescue with RC5B substitution line of spring wheat, at Edmonton Research Station in 1996 in days to heading, * and ** indicates significant difference at 5 and 1 percent level respectively.

Source	DF	Mean Square	F Value	Pr > F
rep	1	14.40	6.13	0.0228*
sdate	9	13.54	5.77	0.0007**
cult	1	96.10	40.94	<0.0001**
sdate x cult	9	2.16	0.92	0.5305
Error	19	44.60	2.34	
Corrected Total	39			

Table 7.3. Analysis of variance comparing Cadet with CR5D substitution line of spring wheat, at Edmonton Research Station in 1996 in days to heading, * and ** indicates significant difference at 5 and 1 percent level respectively.

Source	DF	Mean Square	F Value	Pr > F
rep	1	40.00	18.54	0.0004**
sdate	10	46.00	21.32	<0.0001**
cult	1	0.10	0.05	0.8319
sdate x cult	9	2.10	0.97	0.4911
Error	19	41.00	2.15	
Corrected Total	40			

Examples of Duncan's Multiple Range Test showing temperature differences in 1996, 1997 and 1998 during the study period..

Table 7.4. Duncan's Multiple Range Test for average air temperature, showing differences in each year studied. Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	year
A	14.47	120	98
B	11.72	120	97
B	10.88	120	96

(* at significant level of 0.05)

Table 7.5. Duncan's Multiple Range Test showing differences in days to heading for Rescue in growth chamber and for in the field, in 1997 and 1998. Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	year
A	73.40	10	GC
B	55.24	92	97
B	54.71	34	98

(* at significant level of 0.05)

Table 7.6. Duncan's Multiple Range Test showing differences in days to heading for Cadet in growth chamber and for in the field, in 1997 and 1998. Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	year
A	97.90	10	GC
B	62.63	93	97
B	60.59	34	98

(* at significant level of 0.05)

Examples of Correlation matrix showing relationship between GDD, PTU and PF.

Table 7.7. Correlation matrix determining the relationship between the three variables growing degree-days (GDD), photo-thermal units (PTU) and photon flux (PF) for cultivar Rescue in 1997.

The CORR Procedure			
3 Variables: PF GDD PTU			
Pearson Correlation Coefficients, N = 47			
Prob > r under H0: Rho=0			
	PF	GDD	PTU
PF	1.000	0.763 <0.0001	0.714 <0.0001
GDD	0.763 <0.0001	1.000	0.994 <0.0001
PTU	0.714 <0.0001	0.994 <0.0001	1.000

Table 7.8. Correlation matrix determining the relationship between the three variables growing degree-days (GDD), photo-thermal units (PTU) and photon flux (PF) for cultivar Cadet in 1997.

The CORR Procedure			
3 Variables: PF GDD PTU			
Pearson Correlation Coefficients, N = 38			
Prob > r under H0: Rho=0			
	PF	GDD	PTU
PF	1.000	0.569 0.0002	0.525 0.0007
GDD	0.569 0.0002	1.000	0.997 <0.0001
PTU	0.525 0.0007	0.997 <0.0001	1.000

An example of Duncan’s Multiple Range Test showing seeding date effect.

Table 7.9.Duncan's Multiple Range Test for days to heading in 1996 in Cadet/CR5B showing significant differences in multiple seeding dates. (Means with the same letter are not significantly different).

Duncan Grouping			Mean	N	sdate
	A		66.750	4	1
	A				
B	A		65.500	4	4
B					
B	C		64.000	4	5
B	C				
B	C	D	63.500	4	2
	C	D			
	C	D	62.500	4	3
	C	D			
E	C	D	62.000	4	6
E	C	D			
E	C	D	62.000	4	8
E	C	D			
E	C	D	61.750	4	7
E		D			
E		D	61.000	4	9
E					
E			59.750	4	11
	F		55.000	1	10

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